Effect of Acupuncture on Artery Elasticity for Atherosclerosis: A Randomized Controlled Trial

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Abstract

Background

Atherosclerosis (AS) is a chronic arterial disease. Atherosclerosis related diseases, like myocardial infarctions (MI) and strokes have the highest mortality and disability rate. However, limited evidence verified the effects of acupuncture on arterial stiffness for subclinical atherosclerosis. We hypothesized that acupuncture could improve arterial stiffness in subclinical atherosclerosis and resist plaque progression. The aim of this study is to assess the effect of acupuncture on arterial elasticity via ultrafast pulse wave velocity (ufPWV) and explore the effect of acupuncture on lipid level and platelet function for subclinical atherosclerosis patients.

Methods

This was a randomized parallel controlled trial included 44 patients. Patients were assigned in a 1:1 ratio to acupuncture group and sham acupuncture group. Patients completed 24 treatments in total within 12 weeks of intervention. The primary outcome was ultrafast pulse wave velocity (ufPWV) assessed after every 4-weeks treatment; the secondary outcomes were carotid intima-media thickness (cIMT), blood lipid levels, fibrinogen (FIB) and blood platelet. Intention-to-treat (ITT) principle was applied and data sets were analyzed using SPSS 20.0 software.

Results

Among the 44 randomly assigned patients, changes of right-side BS value in TA group (0.841) at week 12 were greater than SA group (-0.189), with a mean difference of 1.030 (95% CI, 0.320, 1.739; P=0.006). Similar results were observed in right-side ES, left-side BS, left-side ES at week 12. As to secondary outcomes, compared with SA group(1.08mm), the TA group(0.98mm) showed a significant decline in mean of left-side IMT at week 12. (Z= -2.118; P=0.034). There were no serious adverse events.

Conclusions

Among patients with Carotid intima-media thickening, both-side carotids arterial elasticity is significantly improved after 12-week acupuncture compared with sham acupuncture. The effects of acupuncture are more noticeable at week 12 during end-systole.

Trial registration

The trial was registered at http://www.chictr.org.cn (NO. ChiCTR1900025551, 31/08/2019)

Background

Atherosclerosis (AS) is a chronic arterial disease. Main pathological features are thickening of the arterial intima-media and the formation of plaques. Atherosclerosis related diseases, like myocardial infarctions

(MI) and many strokes, as well as disabling peripheral artery diseases have the highest mortality and disability rate [1]. The development of atherosclerosis is a complicated process. inflammation, oxidative stress reaction and hemodynamic factors could slowly damage endothelial cells functions, thus attract low-density lipoprotein (LDL), cholesterol (CT) and platelets stagnate to the local damaged sites in early subclinical atherosclerosis [2, 3].

In current review [4], plaque progression is an essential intermediary step linking early subclinical atherosclerosis to an acute plaque rupture event. Arterial stiffness and endothelial dysfunction are early manifestations of hypertension and subclinical atherosclerosis. As reported [5], arterial stiffness and endothelial dysfunction are related to declined cerebral blood flow, the presence of intracranial stenotic plaque and greater volume of white matter hyperintensity (WMH). Therefore, early detection of subclinical atherosclerosis and restriction of its progression by timely intervention could constitute an important step toward cardiovascular disease prevention. It is recommended that management approaches for peripheral artery disease need earlier diagnosis and intervention [6]. A systematic review compared pulse wave velocity (PWV) in subjects with and without statins has shown that short-term statin therapy has a favorable effect on improvement of arterial stiffness compared to placebo [7].

Acupuncture is considered a beneficial alternative treatment for hypertension and chronic stable angina with little adverse effects [8].Acupuncture also reduced brachial and aortic blood pressure (BP), wave reflection and arterial stiffness in middle-age hypertensive individuals [9]. However, limited evidence exists regarding the effects of acupuncture on arterial stiffness of subclinical atherosclerosis and further investigation is wanted. Therefore, this trial aims to assess effect of acupuncture on artery elasticity for atherosclerosis. We hope acupuncture could improve arterial stiffness in subclinical atherosclerosis and resist plaque progression.

The objective of this study is to assess the effect of acupuncture on arterial elasticity via ultrafast pulse wave velocity(ufPWV) and explore the effect of acupuncture on lipid level and platelet function for subclinical atherosclerosis patients.

Methods

Trial design

The study was a single-blind, double-arm, parallel-design RCT and was randomized into a TA group and a SA group two groups in a 1:1 ratio. The following is a flow chart (Figure 1).

Participants

Recruitment advertisements were disseminated via social media and Outpatient departments in the hospital. Interested patients could consult us by provided phone number or Wechat account. A research assistant would explain the research contents to patients and get the informed consent form signed; Subsequently, the patients would be assigned to undergo carotid ultrasound examination for free. After the screening, Eligible patients would be included according to ultrasound results and demographic information (including age, gender, BMI, blood pressure, whist line, smoking habit, comorbidity and family history). According to SAGER guideline [10], the results and data analysis on gender are not involved in this trail, gender distribution will be reported in order to avoid selective bias only.

Eligibility criteria: Carotid ultrasound examinations (at least one side IMT≥1.0 mm, and both side IMT≤1.5 mm) were required for eligibility before inclusion. To be considered eligible, patients had to comply with the following: Age: 20 -70 years old; Volunteer to take part in the study and signed informed consent form; Good understanding of the trail; Without any other treatment for atherosclerosis in the past 2 weeks (i.e., asprin, plavix, fibrates, statins etc.).

Exclusion criteria

Risk of 10 years' cardio-cerebrovascular disease≥20% [11]; History of acute cerebrovascular events, or severe trauma or major surgery, other serious diseases; Mental disorders; Skin diseases and bleeding disorders; Fear of acupuncture; Pregnant women or lactating women; Severe diabetes, skin irritation, or acute skin lesion.

Randomization

Patients who meet the inclusion criteria will be randomly assigned to TA group and SA group in a 1:1 ratio.

Sequence generation: Randomization was conducted by SPSS software (20.0 ver. IBM SPSS Statistics, IBM Crop, Somers, New York, USA). The allocation sequence was computer-generated with RV. UNIFORM (0,100), set seed=20190901.

Implementation: The random numbers and random cards were produced and stored by Guangdong Provincial Hospital of Traditional Chinese Medicine, Acupuncture Research Centre. Each randomly generated serial number was put into an opaque envelope. These envelopes were allocated to corresponding patients by researchers according to sequential order after inclusion.

Blinding

Considering the specificity of the acupuncture treatment, this trial cannot blind the acupuncturists. Each stage including recruitment, intervention, sample collection, ultrasound examination and follow-up, will be carried out independently by different staffs. Patients, data collectors, statistical analysts and ultrasound physicians were all blinded in this trial.

Ethics

Patients were recruited in Guangdong Provincial Hospital of Traditional Chinese Medicine with the approval of the local Clinical Ethics Committee (YF2019-071-01). Researchers have made sure every patient has a well-grounded understanding of the research contents and get informed consent before inclusion. All collected data from patients including photographs, hard-copy reports and spreadsheets are locked with limited access. The trial was registered at http://www.chictr.org.cn (NO.ChiCTR1900025551,31/08/2019).

Interventions

Patients were randomly divided into two groups: TA group and SA group. Each group's acupuncture points were the same. The selection and location of acupoints were consistent with the WHO acupuncture point positioning criteria (As shown in the table and figure of Supplement 3 eAppendix 2) [12]. The interventions were implemented by 4 experienced and trained acupuncturists under the Clinical Research Acupuncture Operation Standard (STRICTA) [13]. Patients in both groups received a total of 24 treatments twice per week for 12 weeks.

TA group

Before treatment, patients were asked to have a rest in a warm and comfortable environment. The acupuncturist sterilized his hands and patient's skin with 75% alcohol. Next, the acupuncturist located acupuncture points and pasted customized foam pads (1*1*1 cm^3 in size) with a hollow tube on the skin. Hwato brand disposable sterile needles (size 0.25*25 mm) were used to pass the tube and then penetrate into skin through the foam pad in the order of Neiguan (PC6), Yanglingquan (GB34), Renying (ST9), Baihui (DU20) and Yintang (EX-HN3). The depth was between 0.5-1.0 cm according to patients' body shape. The needles were retained for 30 minutes. Finally, the needles were removed without bleeding.

SA group

The environment demand, acupoints, procedures, retention time and acupuncturists were the same as TA group. After sterilization, the acupuncturists covered the points with the same foam pads. Disposable sterile blunt needles (0.25*25 mm) that could not pierce the skin were applied in SA group (As shown in the table and figure of Supplement 3 eAppendix 2). Although blunt needles were inserted through the hollow tube and the foam pad and touched the patient's skin, they could not puncture the skin surface. Therefore, the needles only produced a tingling sensation which was similar to true acupuncture.

Usual care

Patients in both groups received the healthy diet and lifestyle education during treatment periods according to 2020 Guidelines for primary prevention of cardiovascular disease in China [14]. Patients were allowed to receive treatments that were not related to atherosclerosis or other cardiovascular diseases and recorded in CRFs truthfully.

Outcomes

Primary outcomes

The primary outcome was ultrafast pulse wave velocity (ufPWV), which is a shock wave caused by blood flow along the aorta to peripheral arteries during the contraction and dilatation phases of cardiac ejection. The primary outcome was measured by French Supersonic Aixplorer ultrasonic diagnostic instrument with the SL10-2 probe, frequency 2-10 Hz, vascular PWV mode. The sonographer selected 1-1.5 cm below the carotid bifurcation of the common carotid artery as measurement site. The indicators include the pulse wave velocity at begin-systole (BS, m/s) and at end-systole (ES, m/s), as well as the standard deviation (Δ±). If the standard deviation is more than 20% of the corresponding speed, the operation is deemed invalid, according to UFPWV operating guideline from Supersonic Aixplorer Company (details in supplement 4). Both sides carotid arteries were measured at least three times and then the researcher recorded the average PWV value by Excel.

Secondary outcomes

Carotid intima media thickness (cIMT): cIMT is a repeatable and convenient indicator of atherosclerosis as well as a vital indicator for predicting brain and cardiovascular events. According to Chinese Guideline for Ultrasound Examination of Blood Vessels, we defined IMT≥1.5 mm as local plaque formation. Intima - media thickened (at least one side IMT≥1.0 mm, and both side IMT≤1.5 mm) were required for eligibility before inclusion. Patients underwent IMT examinations before the first treatment and then at 4 weeks, 8 weeks and 12weeks.

Lipid level: lipid level indicators included cholestenone (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and apolipoprotein (Apoa). The tests will be carried out by a third party, namely, the Department of Examination of Guangdong Provincial Hospital of Chinese Medicine.

Blood platelet (PLT) and fibrinogen (FIB): Blood platelet (PLT) and fibrinogen plays a vital role in endothelial impairment and plaque formation. Researches suggested that high PLT level patients were more likely to suffer from atherosclerosis or stroke. Fibrinogen is a Blood coagulation functional protein synthesized mainly by liver cells and is the most abundant coagulation factor in plasma. Fibrinogen is an important substrate and key step in thrombosis, and can stabilize the thrombosis structure. High fibrinogen is an important risk factor for various thrombotic diseases including atherosclerosis

Demographic and clinical baseline information at baseline

From patient record: age, gender, family history of cardiovascular disease, co-morbidity, smoking habit and all medication.

Measurements: BMI, blood pressure, waistline.

Sample size

As no clinical studies related to this study were found during the review of previous studies so far, at least 40 participants are required with a 1:1 ratio for two groups. Considering the maximum 15% dropout rate, 46 participants will be included.

Statistical analysis

ITT principle was applied to all participants who have received at least one treatment. As uncompleted patients, missing data was imputed using the last-observation-carried-forward method. Data statistics were runed with SPSS 20.0 software (IBM SPSS Statistics, IBM Crop, Somers, New York, USA) with a set of 95% confidence interval and 2-sided significance level of less than 0.05.

For baseline data, the consistency between two groups will be compared by T test or Mann-Whitney U test. Continuous variables (age, BMI, blood pressure, waistline) were presented by mean and standard deviation if meet normal distribution, otherwise would be presented by mean (median, range) and categorical variables (gender, family history, co-morbidity, smoking habit and medication) were presented by composition ratio. For primary and secondary outcome, t test was used if continuous variables conformed to the normal distribution and uniform variance. Otherwise, we used corrected t-test or two-sample nonparametric tests. Categorical variables were analysed by chi-square test or Fisher’s exact test. When *P* is less than 0.05, it will be defined as having a significant difference. The same approach was used for changes in PWV and cIMT at weeks 4, 8, and 12.

Results

Participants and baseline Characteristics

Since September 2019 to June 2020, 108 patients have been screened ultrasound from clinical recruitment, among them 54 patients were excluded for not meeting diagnostic criteria, 8 were excluded for not meeting eligibility criteria, 2 declined to join in. Amount to 44 patients (mean age, 61.29 years,31.8% (14 of 44) men) were enrolled. 2 in TA group and 1 in SA group were withdrawn due to protocol violation. Eventually 41 patients (21 in TA group and 20 in SA group) with baseline data were included in the full analysis set, and 27(65.85%) participants completed the study (Figure 1). 4 patients' PWV data in TA group at week 4 were missing due to device malfunction. Baseline demographic and clinical characteristics were similar between groups(P>0.05) as shown in Table 1.

Table 1
Baseline Characteristics of the Intention-to-Treat Population

Characteristic	**Acupuncture Group (TA)** **(n = 21)**	**Sham acupuncture Group (SA)** **(n = 20)**
Age, mean (median, range)	61.38(61.70, 48-69)	61.20(60.50, 52-69)
Gender, n (%)		
Male	6(28.6)	8(40)
Female	15(71.4)	12(60)
BMI, mean±SD	21.815±2.1834	22.251±2.3284
Systolic pressure, (mmHg), mean (median, range)	116.29(120,104-140)	121.45(120,110-140)
Diastolic pressure, (mmHg), mean±SD	75.76±6.782	76.50±8.432
Waistline(cm), mean (median, range)	81.67(82,72-88)	80.90(79,72-100)
family history of cardiovascular events, n (%)		
Yes	2(9.5)	4(20)
No	19(90.5)	16(80)
Smoke habit, n (%)		
Yes	5(23.8)	4(20)
No	16(76.2)	16(80)
Co-morbidity, n (%)		
Hypertension		4(20)
Hyperlipidemia	3(14.3)	2(10)
Diabetes	2(9.5)	4(20)
Hyperuricemia		1(5)

[a] BMI: Body Mass Index; IMT: Intima-media Thickness; PWV: Pulse Wave Velocity; TA: true acupuncture; SA: sham

acupuncture; BS:(Beginning of contraction Speed); ES:(End of contraction Speed); SD: standard deviation.

[b] There were no significant differences (P >0.05) between TA group and SA group of baseline characteristics.

Characteristic	Acupuncture Group (TA) (n = 21)	Sham acupuncture Group (SA) (n = 20)
IMT (mm)		
Right, mean±SD	1.01±0.180	1.00±0.145
Left, mean (median, range)	1.02(1.00,0.7-1.3)	1.11(1.15,0.8-1.4)
PWV, (m/s)		
BS, right, mean±SD	6.073±1.4842	5.747±1.1737
ES, right, mean±SD	8.673±1.4951	8.289±1.3886
BS, left, mean (median, range)	6.591(6.870,4.100-9.443)	5.902(5.419,3.937-10.090)
ES, left, mean±SD	9.456±1.9451	8.733±2.0321
[a] BMI: Body Mass Index; IMT: Intima-media Thickness; PWV: Pulse Wave Velocity; TA: true acupuncture; SA: sham		
acupuncture; BS:(Beginning of contraction Speed); ES:(End of contraction Speed); SD: standard deviation.		
[b] There were no significant differences (P >0.05) between TA group and SA group of baseline characteristics.		

Primary outcome

Mean of PWV (Both sides of BS and ES value) at every timepoint are shown in Table 2 and Figure 2. Right-side BS value decreased by 13.8% from baseline (6.073 m/s) to week 12 (5.232 m/s) in TA group, increased by 3.3% from 5.747 m/s to 5.936 m/s in SA group; The right-side ES value decreased up to 19.2% in TA group, decreased up to 6.7% in SA group; the left-side BS value in TA group decreased by 12.1% from 6.591m/s to 5.796 m/s in TA group, while increased up to 6.8% in SA group; the left-side ES value decreased up to 15.1% from 9.456 m/s to 8.031m/s in TA group, and the left-side ES value decreased up to 6.0% in SA group respectively.

Results in details were observed in right-side ES value and left-side BS/ ES value as shown at Table 2. Although the means of both sides PWV value over time between TA group and SA group were similar (*P*>0.05 at week 0, 4, 8, 12), changes of right-side BS value in TA group (0.841) at week 12 were greater than SA group (-0.189), with a mean difference of 1.030 (95% CI, 0.320, 1.739; *P*=0.006(Figure 3 and Supplement 3 eAppendix 3). Similar results were observed in right-side ES, left-side BS, left-side ES at week 12 (Figure 3).

Table 2

Outcomes of the Intention-to-Treat Population

Outcomes	Acupuncture Group (n = 21)	Sham acupuncture Group (n = 20)	*P* Value
Primary outcome			
PWV, BS(m/s), right			
Week 0, mean±SD	6.073±1.4842	5.747±1.1737	0.442
Week 4, mean±SD	5.645±1.3151	5.619±1.2329	0.948
Week 8, mean±SD	5.138±0.9793	5.618±1.3282	0.194
Week 12, mean (median, range) [b]	5.232（5.213，3.715-7.903）	5.936（5.736，3.420-9.783）	0.124
PWV, ES(m/s), right			
Week 0, mean±SD	8.673±1.4951	8.289±1.3886	0.400
Week 4, mean±SD	8.338±1.6786	7.936±2.1009	0.502
Week 8, mean±SD	7.008±1.5819	7.736±2.1807	0.226
Week 12, mean±SD [b]	7.219±1.7543	8.204±2.2844	0.128
PWV, BS(m/s), left			
Week 0, mean (median, range)	6.591（6.870，4.100-9.443）	5.902（5.419，3.937-10.090）	0.167
Week 4, mean±SD	7.936±2.1009	6.303±1.2346	0.127
Week 8, mean±SD	6.353±1.2180	6.105±1.5323	0.568
Week 12, mean±SD [b]	5.796±1.3058	6.045±1.2195	0.525
PWV, ES(m/s), left			
Week 0, mean±SD	9.456±1.9451	8.733±2.0321	0.251
Week 4, mean±SD	8.349±2.0829	8.350±1.6153	0.998
Week 8, mean±SD	8.679±1.7034	8.208±1.9111	0.410
Week 12, mean±SD [b]	8.031±1.6433	8.397±1.7087	0.490
Secondary outcome			
IMT (mm), right			
Week 0, mean±SD	1.01±0.180	1.00±0.145	0.926

Week 4, mean±SD	1.01±0.170	0.99±0.127	0.605
Week 8, mean (median, range)	1.02(1.00,0.8-1.4)	0.97(1.00,0.6-1.2)	0.454
Week 12, mean (median, range)	1.00(1.00,0.7-1.4)	1.00(1.00,0.6-1.4)	0.873
IMT (mm), left			
Week 0, mean (median, range)	1.02(1.00,0.7-1.3)	1.11(1.15,0.8-1.4)	0.068
Week 4, mean (median, range)	1.019(1.00,0.7-1.3)	1.10(1.10,0.8-1.3)	0.146
Week 8, mean (median, range)	0.99(1.00,0.7-1.3)	1.09(1.1,0.8-1.3)	0.072
Week 12, mean (median, range)	0.98(1.00,0.7-1.3)	1.08(1.10,0.7-1.3)	0.034 [c]
TG (mmol/L), mean (median, range)			
Week 0	2.145(1.40,0.45-16.60)	1.146(0.89,0.48-2.75)	0.076
Week 4	1.498(1.22,0.68-4.04)	1.327(0.91,0.55-3.47)	0.137
Week 8	1.455(1.25,0.56-2.99)	1.473(1.16,0.56-3.03)	0.686
Week 12	1.482(1.28,0.71-2.73)	1.213(1.08,0.55-2.73)	0.130
TC (mmol/L), mean±SD			
Week 0	5.293±1.2248	5.093±0.9253	0.560
Week 4	5.257±1.2087	5.080±1.0547	0.621
Week 8	5.223±1.0219	4.943±0.9987	0.381
Week 12	5.105±0.9651	4.845±0.8249	0.362
LDL (mmol/L), mean±SD			
Week 0	3.520±1.2253	3.355±1.0036	0.641
Week 4	3.716±1.1569	3.323±1.1558	0.283
Week 8	3.610±0.8841	3.175±0.9844	0.144
Week 12	3.518±0.8309	3.1580±0.8922	0.189
HDL (mmol/L), mean±SD			
Week 0	1.410±0.2645	1.637±0.4575	0.058
Week 4	1.422±0.2901	1.621±0.4618	0.105
Week 8	1.430±0.2794	1.634±0.4550	0.090
Week 12	1.390±0.2642	1.618±0.4369	0.048 [c]

Apoa(mg/L), mean (median, range)			
Week 0	149.33(133,23-413)	224.30(126.5,56-1104)	0.774
Week 4	147.29(120,23-341)	242.05(131,55-1160)	0.449
Week 8	154.81(136,14-376)	257.05(179,43-1202)	0.419
Week 12	160.48(135,30-389)	258.60(165,41-1229)	0.382
Platelet (×10^9/L), mean±SD			
Week 0	251.71±69.073	243.10±36.821	0.624
Week 4	243.33±60.590	246.90±41.122	0.827
Week 8	256.57±81.814	245.90±41.122	0.604
Week 12	253.24±79.606	246.50±37.216	0.733
FIB (mmol/L)			
Week 0, mean (median, range)	3.834(3.61,2.99-5.83)	3.381(3.44,2.65-4.10)	0.057
Week 4, mean±SD	3.731±0.6197	3.522±0.5451	0.261
Week 8, mean±SD	3.541±0.5884	3.422±0.5309	0.501
Week 12, mean (median, range)	3.410(3.29,2.50-4.73)	3.564(3.43,2.69-5.68)	0.794

[a] TC: Cholestenone; TG: Triglyceride; HDL: High-density Lipoprotein cholesterol; LDL: Low-density Lipoprotein cholesterol;

Apoa: Apolipoprotein a; PLT: Blood Platelet; FIB: Fibrinogen.

[b] A statistical between-group difference with the change of PWV at week 12 ($P<.05$).

[c] A statistical between-group difference with the mean of left cIMT and HDL at week 12 ($P<.05$)

Secondary outcome

IMT. At the end of week0, 4, 8, there are no differences were observed between the TA group and the SA group in IMT (P >0 .05 for all comparisons) except for mean of left-side IMT at week 12. Compared with SA group(1.08mm), the TA group(0.98mm) showed a significant decline in mean of left-side IMT at week 12. (Z= -2.118; P=0.034; Table 2).

Lipid level. TG, TC, HDL, LDL and Apoa were observed during intervention period at week 0, 4, 8 and 12. At the end of treatment, no differences were observed between TA group and the SA group in TG, TC, LDL and Apoa level (*P* >0 .05 for all comparisons). Significant differences in HDL between the two groups were only found at week 12 (95% CI, -0.455, -0.017; *P*=0.048; Table 2). However, changes of HDL after 12 weeks treatment show no significant difference between TA group and SA group (Supplement 3 eAppendix 4).

Coagulation function. There is no difference between TA group and the SA group in FIB and PLT level at every timepoint (*P* >0 .05 for all comparisons, Table 2).

Safety analysis

In total of five patients (3 in the TA group and 1 in the SA group) reported slight

adverse effects during 12 weeks treatment. Three patients in TA group had subcutaneous haemorrhage around the needle insertion area. One patient in SA group complained of mild skin allergy and itchy induced by stickers on the foam pads. All adverse effects were mild and none required special medical interventions.

Discussion

This randomized, sham controlled clinical trial found that acupuncture therapy was more effective than sham acupuncture in improving carotid arterial pulse wave velocity, which indicated improvement in carotid arterial stiffness and elasticity. Acupuncture effects were more significant after the 12-weeks intervention. However, lipid level and Coagulation function show little difference over the treatment period.

Interpretation

The mean PWV of 42 patients with mild carotid intimal thickening at baseline respectively was BS, right (5.91m/s); ES, right (8.49m/s); BS, left (6.26m/s) and ES, left (9.10m/s). Refer to the latest achievement, the normal carotid PWV range of Chinese adults is about BS, right (5.29m/s); ES, right (6.80m/s); BS, left (5.63m/s) and ES, left (7.28m/s) [15], which are congruent with another research in Europe [16]. We found changes of arterial stiffness was more aggressive than intima-media thickness with age. IMT assesses structural changes in the vasculature, while PWV reflects changes of dynamic vasomotor function. Previous study presented the lifetime trajectories of arterial stiffening from adolescents to the elderly, suggesting increases in PWV at an early age are associated with an increased future CVD risk [17]. Therefore, PWV may be more sensitive and suitable for predicting CVD risk in early subclinical atherosclerosis, and assess prevention effectivity momentarily.

In terms of PWV outcome, although our study showed no significant difference in the mean PWV value between TA group and SA group, the changes of both-sides PWV value were statistically significant at week 12 between two groups. As reported above, PWV value in TA group decreased up to 19.2%, and decreased up to 6.0% in SA group. Our findings were similar to a previous clinical trial for hypertension [9]. Vascular aging and atherosclerosis progression is an insidious and continuous process, so we believed 12.1–19.2% improvement in PWV over 12-weeks acupuncture treatment would constitute a clinically meaningful outcome. It can be concluded that acupuncture treatment can improve the arterial elasticity of carotid.

Our trial also observed that bilateral carotids elasticity is different. No matter in normal population or patient with intimal thickening, left-side mean PWV is higher than right-side. We speculated this difference may attribute to difference anatomical structures of both-side carotids. Previous study has explored different flow pattern and structural changes at carotid bifurcation in hypertensive cynomolgus monkeys, and hemodynamic alterations could cause endothelial disarray and leucocytic adherence in early atherogenesis [18]. Moreover, patients with cardiogenic embolisms were more likely to have right-sided lesions, whereas patients with aortogenic embolisms more frequently had left-sided lesions [19]. PWV changes after 12-week acupuncture treatment were similar between right side and left side carotid (19.2% and 15.1% respectively), which suggested no matter hemodynamic alterations or structural difference, acupuncture may be helpful to prevent both sides atherogenesis.

Furthermore, the findings of the current study demonstrated that improvement in ES is more significant than BS over intervention period. One previous research on quantification of aortic stiffness using magnetic resonance elastography (MRE) confirmed that Mean μMRE was higher during end-systole when compared to end-diastole [20]. This suggested PWV changes are different over cardiac cycle. BS refers to PWV during begin-systole, which is characterized by vascular smooth muscle cell (VSMC) constriction. VSMC plasticity and signaling are highly relevant to the physiology of vascular aging [21]. ES refers to PWV during end-systole, which is associated with aerial compliance to a greater extent, and endothelial cell compliance may serve as a protective mechanism that maintains blood vessel integrity in vivo [22]. These findings suggested that acupuncture may have a preferred effect on ameliorating endothelial cell compliance than vascular aging resistance, and arterial compliance may identify subjects with extensive subclinical atherosclerosis [23].

Although PWV showed a decline trend at week 4 and week 8, a statistically significant between-group difference in favor of acupuncture was observed only at week 12. This supported that acupuncture may have an accumulated effect on long term prevention of atherosclerosis. We set twice a week as treatment frequency in this trial [24], and we thought this is a proper interval and easy to maintain which is crucial in chronic progressive disease like atherosclerosis. However, the long-term effect of acupuncture like whether such PWV improvement could reduce CVD events need to be verified.

Despite the exact mechanism of acupuncture on vascular remains unknown, we speculate that PWV improvement in our trial were mainly due to hemodynamic responses and autonomic nerves system (ANS) activity associated with de-qi sensations induced by acupuncture [25, 26]. Several clinical trials have proved hemodynamic and ANS activity changes of acupoints [27–29]. For example, PC6 needling produced changes in both large artery stiffness and small arteries; ST9 could regulate BP via induing hemodynamic changes and pressure sensor. Thus, our trial may attach PWV improvement by changing flow patterns and autonomic nerves system (ANS) activity.

In terms of secondary outcomes, only mean of left IMT and HDL at week 12 showed statistically significant between two groups. However, the changes of IMT and HDL showed no statistically difference (Supplement 3 eAppendix 4, Table 3). Thus, we inferred that cIMT and HDL changes between two groups have no clinical significance. IMT is a classic outcome for predicting cardiovascular outcomes. It has been verified that maximum IMT of the internal carotid artery improved the classification of risk of cardiovascular disease in the Framingham Offspring Study cohort [30]. As other trial reported, carotid IMT has a positive correlation to PWV in type 2 diabetes patients [31]. We suspected IMT assesses structural changes, so IMT changes appears more inconspicuous than PWV improvement. Lipid levels are also necessary to achieve ideal cardiovascular health [32], and previous study indicated that electro-acupuncture at ST40 can lower the levels of serum cholesterol and triacylglycerols [33], so we selected Lipid levels as secondary outcomes. Despite the results show no changes in lipid levels, we speculated this might be associated with different acupoints selection or bias caused by diary. At last, it was expectable that results showed no difference between two groups in FIB and PLT level at every timepoint, because coagulation function changes are usually accompanied by plaque progression or rupture [34], while patients in this trial were at subclinical statue before plaque formation.

Limitations

This study also has limitations. First, in terms of study protocol design, acupoints selection and secondary outcomes selection show little association. For example, previous system review indicated the first three top acupoints of high frequency used in lowering lipid level were Fenglong (ST40), Zusanli (ST36), Sanyinjiao (SP6) [35], which we did not adopt. This might cause unsignificant results in blood lipid outcomes. Second, patients only received cursory healthy diet and lifestyle education during intervention period. However, daily diary influence on lipid level cannot be ignored and we did not record dietary habits and enhance diet control for every patient which may lead to bias. Third, we only included 44 patients in total, larger sample size is necessary for further study. Fourth, because acupuncture treatment should be operated by acupuncturists according to randomized outcome, so acupuncturists could not be blinded. Fifth, because of limited study time allowance, we did not add follow up period. Thus, we are disable to observe long-term effects of acupuncture on arterial stiffness and atherosclerosis progression. Study on relationships between PWV improvement and CVD events is also warrant.

Conclusion

In conclusion, among patients with Carotid intima-media thickening, both-side carotids arterial elasticity is significantly improved after 12-week acupuncture compared with sham acupuncture, the effects of acupuncture are more noticeable at week 12 during end-systole. However, lipid level and coagulation function show negative results. Further studies are warranted to investigate long-term effects of acupuncture, particularly in relationship between PWV improvement and CVD events.

Declarations

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Ethics approval and consent to patients

This study was reviewed by the Clinical Ethics Committee of Guangdong Provincial Hospital of Traditional Chinese Medicine (YF2019-071-01) in accordance with the Declaration of Helsinki. Patient consent were obtained before inclusion.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are not publicly available due further related study necessary, but are available from the corresponding author on reasonable request.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship and publication of this article

Author contribution

Lin Zhao and Jianxing Zhang were responsible for most of the writing and editing of the manuscript. Peng Zhou and Wenbin Fu are the major researchers in this study and participated in the design. Lingcui Meng and Siting Ye were responsible for all ultrasound examination tasks. Junhe Zhou, Huitao Liang and Ting Zhou and were responsible for the recruitment of the participants. Xichang Huang, and Zhiqi Qi were responsible for the data collation and input.

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Figures

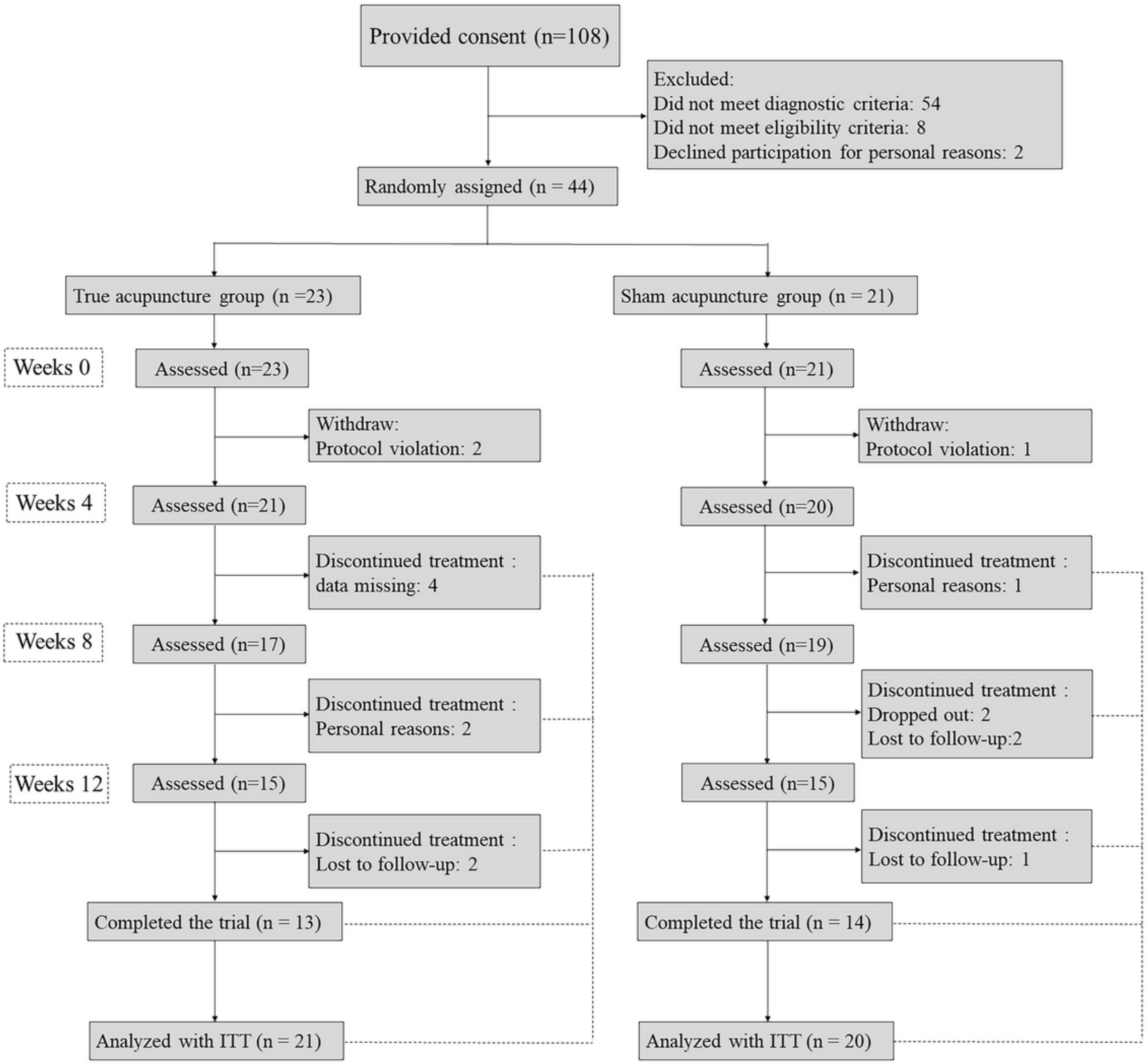

Figure 1

Flow chart, ITT: intention-to-treat; 4 TA patients' PWV data of week 4 missing due to device malfunction.

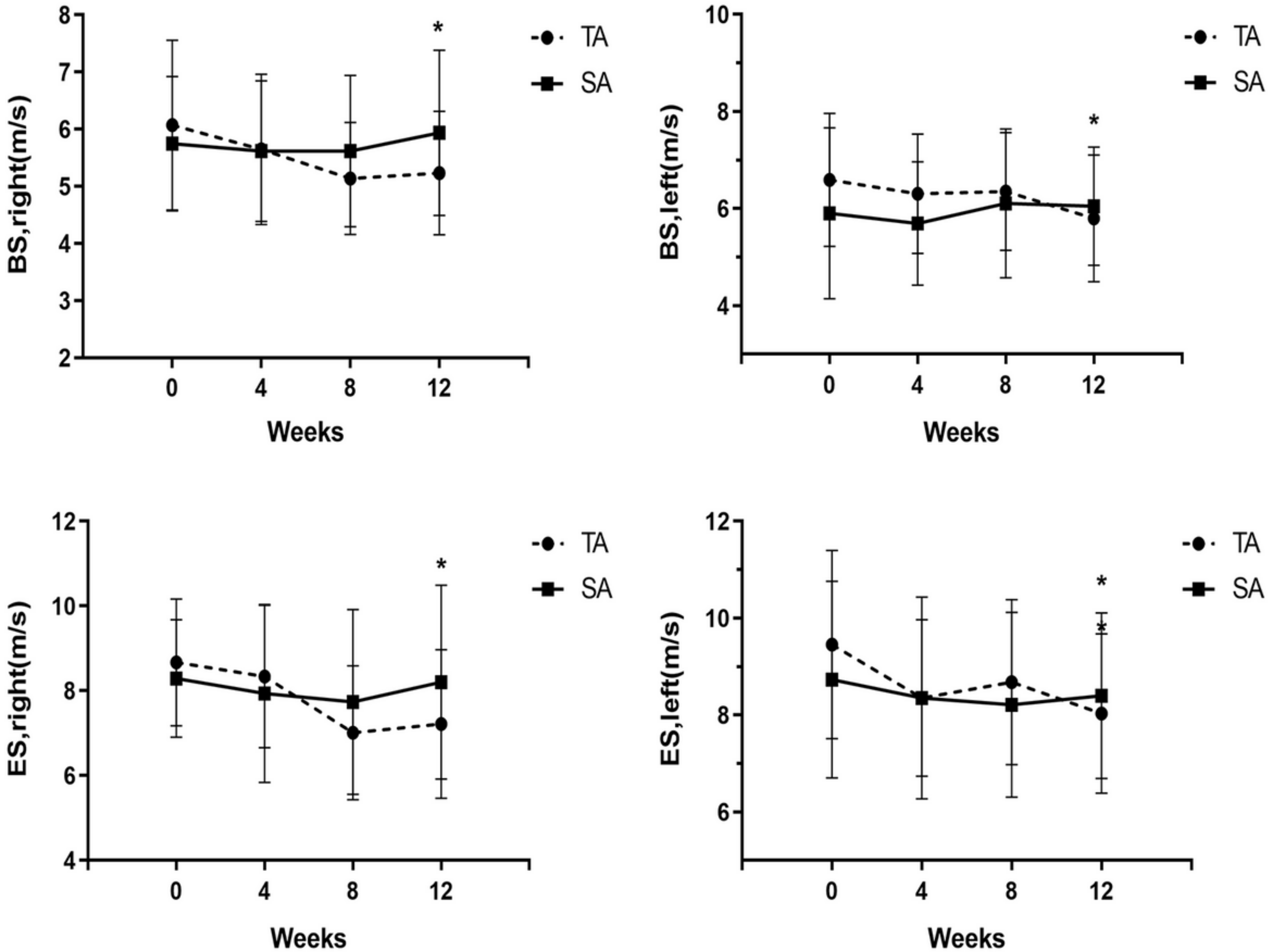

Figure 2

PWV value change at the beginning of carotid artery contraction (BS) and the end of contraction (ES) at each assessment time point.

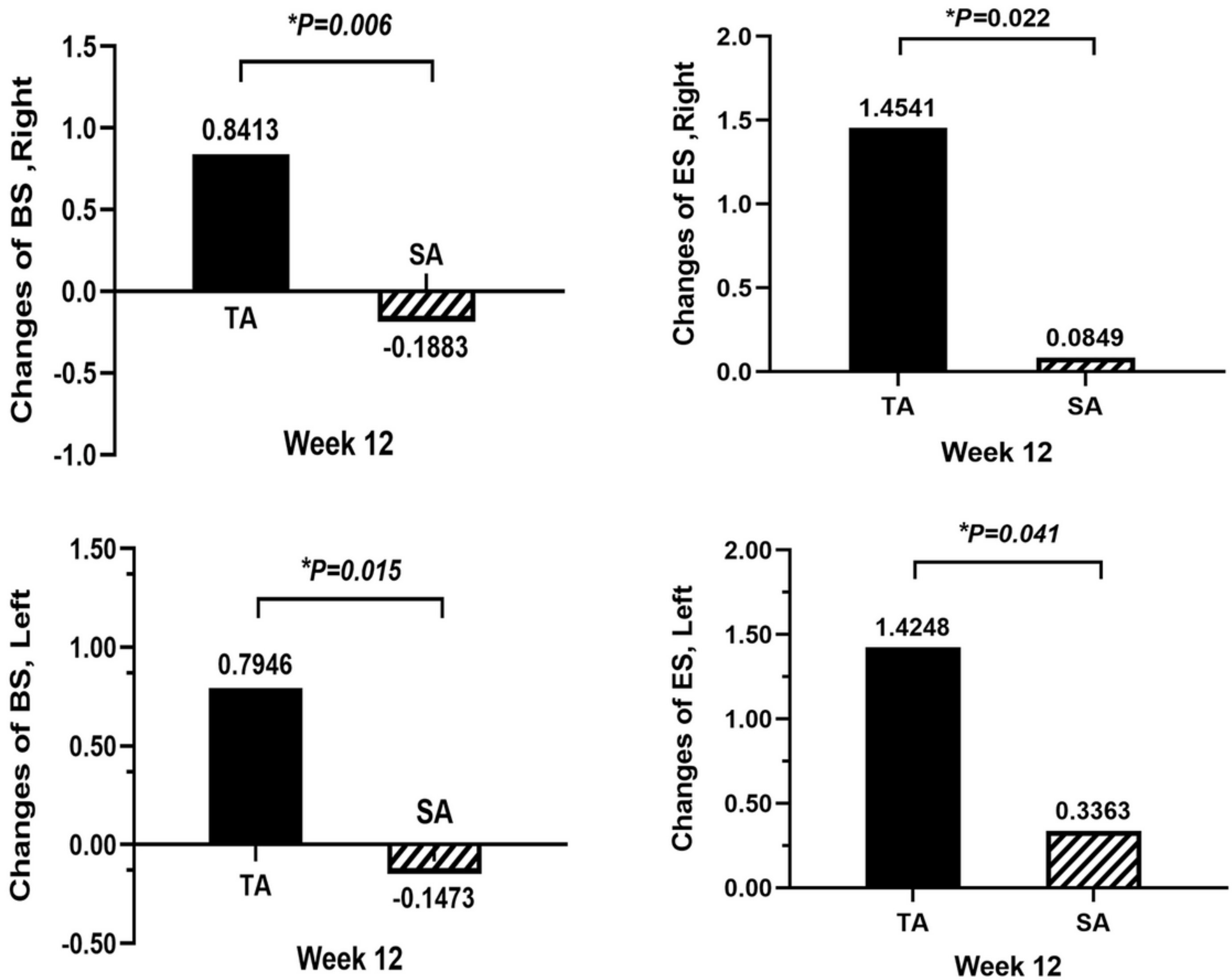

Figure 3

Changes of PWV (both sides BS and ES value) at week 12.

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- supplement2WHOprotocol.docx