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Acupuncture Combined with Hydrotherapy in Diabetes Patients with Mild Lower-Extremity Arterial Disease: A Prospective, Randomized, Nonblinded Clinical Study

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Background: The aim of this pragmatic study was to explore the intervention of acupuncture combined with hydrotherapy and perceived effects in type 2 diabetic patients with recently diagnosed, mild, lower-extremity arterial disease (LEAD) in comparison with a control group.





Material/Methods: One hundred twenty-six diabetes patients who were diagnosed mild LEAD according to ankle-brachial blood pressure index (ABPI) and peripheral neuropathy symptom were randomly assigned to either an experimental (n=64) or control group (n=62). The experimental group attended and completed (1) a 30-min session of acupuncture in certain selected points, and (2) a 30-min hydrotherapy exercise every 2 days for 15 weeks. The outcome parameters were assessed at baseline, after intervention, and at 6-week follow-up.

Results: The intervention was associated with an improvement in leg flow conductance and partial physical capacities, including chair-sit-and-reach, the walking impairment questionnaire (WIQ), and physical component summary score (PCS), compared to the control group. The treatment benefits were sustained throughout the 6-week follow-up endpoint. There was no difference in fasting glucose levels, Hb1Ac, blood pressure, or BMI after the intervention. At the endpoint of 6-week follow-up, acupuncture plus hydrotherapy appeared to reduce inflammatory response by decreasing IL-6, TNF- α , malondialdehyde, and SOD, and increasing glutathione.

Conclusions: Acupuncture plus hydrotherapy, without significant glycemic-controlling effects in the type 2 diabetic patients with mild LEAD, exerts a measurable benefit in disease-specific physical functions and health-related quality of life. Our results suggest that the combined therapy regulates the inflammatory process and oxidative stress and contributes to immune protection.

MeSH Keywords: **Acupuncture • Diabetes • Hydrotherapy • Inflammatory • Lower-Extremity Arterial Disease • Oxidative Stress**

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Background

With the economic globalization, urbanization, and aging population, the incidence of diabetes has progressively increased over the last decade in developed and developing countries [1,2]. With high prevalence of type 2 diabetes worldwide, diabetic complications such as retinopathy, nephropathy, neuropathy, skin ulcer, and atherosclerosis have become a major health problem leading to reduction of life quality and increase in premature death in adults. The clinical and public health importance of peripheral artery disease (PAD), particularly lower-extremity artery disease (LEAD), among the people with diabetes has been increasingly recognized over the last 20 years in China and has become one of the most common forms of peripheral vascular disease in the last decade. The epidemiological evidence of diabetes-induced LEAD is represented clinically as chronic or acute-on-chronic ischemia occurring in the whole or part of an extremity, with resultant numbness, resting pain, and claudication with or without associated ulceration and gangrene. In Western countries, approximately 35.0% of diabetic people aged >40 years have at least 1 LEAD condition, and this rate is higher among persons aged >60 years than among those aged 40–59 years. Of these, about 53% of those with diabetes were symptomatic. In China, multiple complications of LEAD in the diabetic population are the leading cause of non-traumatic amputations. For patients aged over 65 years, diabetes causes a more than 20-fold higher risk for amputation and limb loss.

Based on the traditional theory, the effect of acupuncture has been well documented in studies through targeting an imbalance of *yang* and *yin*, *qi*, and *xue*. Hydrotherapy is widely used for people with some chronic illnesses such as arthritis [3] and OA [4], as well as multiple sclerosis [5], in observational investigations. It is suggested that hydrotherapy can provide pain relief, reduce edema, and alleviate loading pressure on the key joints. It has been recognized that complementary and alternative therapies, including traditional herbal, Tai Chi, massage, and yoga, can be integrated in the management of diabetes mellitus. Several investigations have addressed the effectiveness of complementary methods prescribed in clinical settings [6]. To the best of our knowledge, no data is available evaluating the effect of acupuncture plus hydrotherapy as an intervention in diabetics with LEAD.

Our major hypothesis is that there is a complementary-induced change in physical functions mediated by integrating 2 different alternative actions. If this were the case, combination of acupuncture and hydrotherapy might improve clinical outcomes among diabetes patients with LEAD in addition to routine care.

The secondary aim of this study was to determine if the treatment can rebalance serum inflammatory markers among

diabetics with LEAD. We hypothesized that systemic inflammatory responses could result from acupuncture action in stimulating pitch points of meridians as well as thermal hydrotherapy. Our study explored changes in serum inflammatory markers C-reactive protein (CRP), inflammatory cytokines, and plasma lipid peroxidation mediators [7–16] during the clinical observation in order to elucidate the possible mechanisms of the complimentary effects of these 2 combined therapies.

Material and Methods

The trial design and the participants

This randomized, controlled, single-center trial enrolled patients from November 2014 to March 2015 at Hebei Chronic Disease Rehabilitation Center (HCDRC), Qinhuangdao, China. A senior PMR was selected as the trial examiner, who remained blind to participants' group allocation and carried out all outcome assessments. All the eligible participants were randomly assigned by a blinded senior PMR researcher, using a computer-generated randomized list, to the acupuncture plus thermal hydrotherapy group or the control group.

Diabetes was confirmed if the patient was taking diabetes medications, had a medical report of 6.5% or higher Hb1Ac, or had a record of physician-diagnosed diabetes. LEAD was defined as: 1) PAD (ankle-brachial blood pressure index (ABPI) of <0.9 in either leg), 2) PN (1 or more insensate areas in either foot), 3) self-reported history of a foot ulcer or sore on a leg or foot that took >4 weeks to heal, or 4) observed foot lesions or foot/toe amputation. PAD cases were classified as symptomatic if participants answered "yes" when asked whether they ever had calf pain in either leg while walking. Two hundred thirty-five diabetic type 2 patients aged 62–68 years who were suffering from lower-extremity ischemia were admitted in HCDRC. The clinical diagnosis was according to clinical inclusion criteria: persistently recurring numbness, resting pain and intermittent claudication for at least 2 weeks, and/or foot ulcers or gangrene. All diagnoses were confirmed by Doppler sonography and angiography at recruitment. Exclusion criteria were treatment with another CAM, either current or within the previous 3 months, and cardiovascular emergency, current or within the past 3 months. History of pharmacological treatment was collected. Smoking history was collected because smoking is an important risk factor for peripheral arterial disease. Patients who had never smoked cigarettes daily or quit smoking more than 5 years ago were considered non-smokers, and those who reported current daily smoking or quit less than 5 years were classified as smokers. Alcohol drinking was defined as consumption of 30 g of alcohol per week for 1 year. None of the subjects were on antioxidant supplementation. Data on the optimal ABPI threshold for the diagnosis

of mild LEAD are scarce since different criteria have been used to determine the optimal cutoff value. In current study, the cutoff value for diabetic people with mild LEAD was between 0.74 to 0.90. Furthermore, only those who have symptomatic PAD were recruited as participants in clinical judgement. Symptomatic PAD cases were positive if patients answered 'yes' when asked whether they ever had calf pain in either leg while walking. Since the sensitivity of ABPI could be significantly increased in post-exercise, out patients have been tested ABPI after one minute of treadmill exercise to determine the degree of LEAD.

Sampling

One hundred ninety-three diabetes type 2 patients with mild LEAD were recruited in the clinical trial. Among the 193 accessible patients, 39 did not meet the inclusion criteria and 19 refused participation at recruitment or during the study. According to the study design, the 2 randomized groups had approximately equal medians for basic variables. Informed consent was obtained from each patient before entering the study, which was performed in accordance with National Human Subjects Protection Committee on Clinical Trials and Patient Confidentiality. The study protocol was approved by the Hebei Clinical Ethics Committee. The division of groups was balanced for the severity of LEAD measured and those variables including HbA1C levels and smoking history as well as medicine treatment, using a stratification system for each combination of LEAD levels and variables.

Interventions

We performed an experimental clinical trial with acupuncture plus low-radon hot spring thermal hydrotherapy for the treatment group and a control group. The study was performed from April 2015 through October 2015 in HCDRC, Qinhuangdao, China. The aim and process of the study was explained to all participants and then individual written informed consent was collected. The intervention package for acupuncture plus hydrotherapy was scheduled in the 15 consecutive weeks. The treatment session consisted of 2 sub-sessions: (1) a total of 12 acupoint locations on the body for 30-min acupuncture needling *in situ* in the morning, and (2) a 30-min thermal hydrotherapy in the afternoon of the same day. There was 1 session every 2 days and participants were convened in small groups of 12.

The acupoints used out in the study were Zusanli (ST 36), Taibai (SP 3), Sanyinjiao (SP 6), Fenglong (ST 40), Taixi (KI-3), and Yanglingquan (GB 34). The auxiliary points used were Hegu (LI 4), Quchi (LI 11), Chongyang (ST 42), and Yinglingquan (SP 9) to improve circulation stasis and phlegm; and Bafeng (EX-LE10) and Xiyan (EX-LE5) to improve numbness of the lower extremity. The acupuncture point selection was tailored based

upon the significant communication of *yuan*-source, *zang-fu* organs (*shen*) and *qi* within the key meridians through which dampness, phlegm, and stagnation of blood as well as lower extremities' numbness can be alleviated, and internal organs (e.g., spleen, liver and kidney) are nourished and invigorated.

A licensed and experienced acupuncturist identified and marked each location of the acupoints based upon the traditional method. The precise location of needles was determined by participant's achievement of "De Qi" sensation after a reinforcing-reducing rotation every 6 min performed by the acupuncturist. The manipulation can produce correct effects: a slight depression perceived by the participant as an ache or heaviness in the area surrounding the needle, and a grasping-needle sensation by the acupuncturist.

In a second sub-session treatment, a trainer supervised relaxation exercise for 10 min before and after hydrotherapy. The relaxation regimen was standardized and well instructed by the licensed physiotherapist, focused on the progressive muscle relaxation under guided mediation and progressive stretching and resistance movement. The exercise was designed to enhance the range and power of movement on the elbow, hip, knee, ankle, and foot. The speed and strength were adjusted by the instructor based upon the participants' capabilities. To avoid fatigue, pillows were used to support back, knees, or hip on the mats. The patients then slowly entered the hot spring water at 60–65°C with the water up to the suprastomal notch. For those who had ABPI <0.40, a weighted chair was placed in the water. When immersed in the hot water, the patients kept the upper and lower limbs straight up and allowed a slight movement. At the end of the 30-min immersion, participants slowly stood up and left the pool. All the patients were required to rest for 5–10 min in a thermally neutral room with temperature 25°C before and after the intervention. The main components levels in the hydrotherapy are shown in Table 1.

Treatment was immediately terminated if there was any breathing difficulty or palpitation or intolerable leg/foot pain and the patient would rest in a quiet room. For participants in the control group, the daily routine activities were maintained with no acupuncture or hydrotherapy or body relaxation exercise during the study period. The control group subjects met once a month following up and equivalent data collection timed to match that in the intervention group.

Pre-test and pilot study

A pre-test was administered by assessing physical status and self-report questionnaires before and after the intervention. Brief surveys on 9 type 2 diabetic patients selected were collected before a pilot study to purposively explore interest, motivation, and expected benefits through the treatments.

Table 1. The basic characteristics of the concentrations of main materials in the hydrotherapy.

| Main components | Levels | |
|---------------------------------------|--------|--------|
| Temperature (°C) | 65 | (2) |
| pH value | 7.8 | (0.9) |
| TDS (mg/L) | 1599 | (144) |
| Na+ (mg/L) | 354.7 | (21.5) |
| K+ (mg/L) | 2.98 | (0.11) |
| Mg+ (mg/L) | 5.60 | (0.42) |
| Ca ²⁺ (mg/L) | 8.92 | (1.52) |
| SiO ₄ (mg/L) | 34.3 | (2.8) |
| Cl ⁻ (mg/L) | 107.1 | (9.3) |
| HCO ₃ ²⁻ (mg/L) | 1085.3 | (95.2) |
| SO ₄ ²⁻ (mg/L) | 87.9 | (9.3) |
| Radon (Bq/m ³) | 127 | (11.6) |
| Br ⁻ (mg/L) | 38.2 | (4.9) |
| Fe ²⁺ (mg/L) | 19.1 | (2.2) |
| Sr (mg/L) | 35.3 | (4.8) |
| Li (mg/L) | 1.29 | (0.09) |
| Mn (mg/L) | 1.55 | (0.12) |
| BHBO ₂ | 1.24 | (0.11) |
| Conductivity (μS/cm) | 1907 | (122) |

Data are means (SD); TDS – total dissolved solids.

Afterwards, a pilot study was conducted to identify the weakness and therapeutic benefits for the participants. Accordingly, the limitations of the pilot study were rectified to facilitate the feasibility and logistics of the protocol. All subjects (acupuncture plus hydrotherapy and control) were given a gift certificate for free diabetes consultation and free monitoring of blood glucose levels and dietary recommendations.

Data collection in outcomes

The collection and analysis of all outcomes described in the protocol were conducted by blinded assessment.

The lab examiners, clinical psychologist, and statistic staff were blind to participants' intervention and control groups. The data was recorded in coded documents for the final analysis.

The interview assessment, self-report measure, physical status, and hemodynamic and biochemistry measures were conducted at the baseline, after 15-week hydrotherapy, and at

6-week follow-up time points during subjects' visits at the rehabilitation center. The primary outcomes were: (1) symptomatic LEAD assessment through ABPI, Edinburgh claudication questionnaire (ECQ) evaluation and leg vascular conductance (LVC) values, (2) laboratory physical status through lower-body flexibility and aerobic walking endurance measures, and (3) self-report measures through the walking impairment questionnaire (WIQ), EQ-5D, DASS21, and SF-12.

In brief, ABPI was calculated by dividing the average of systolic blood pressure measured in the dorsalis pedis and posterior tibial artery pressures by the average systolic blood pressure measured in brachial artery pressures in a supine position [17]. It was determined by the following equation: ABPI=ankle systolic blood pressure/brachial systolic pressure. According to ABPI values, the severity of LEAD was classified as normal, mild, moderate and severe degrees. Edinburgh claudication questionnaire (ECQ) was carried out based on a previous study [18–20] for diabetic survey in intermittent claudication. Based on the ECQ, the intermittent claudication with LEAD was defined as grade 1 or grade 2. For leg vascular conductance, the method was based on a previous report [21] which estimated leg vascular conductance using diving leg blood flow by simultaneous measured mean arterial pressure as the following equation: LVC=leg blood flow/mean arterial pressure. Leg blood flow was measured by venous occlusion plethysmography and mean arterial pressure was measured simultaneously beat-to-beat through radial artery applanation tonometry (T-Line system, San Diego, USA). LVC was expressed as milliliters per min per mmHg.

Lower-body flexibility was defined as chair-sit-and-reach in which the distance from the subject's finger tips and toe tips of extended legs. Hip joint flexibility was assessed by the rotation angle from the pelvis to femur as the subject lifting the leg straight up in the supine position. The measures' accuracy and reliability were evaluated elsewhere [22–24]. Aerobic walking endurance was measure according to the description in previous reports [25,26] and adjusted to 6-min walking distance and 4-min walking speed by a standardized protocol.

Self-reported physical symptoms were estimated by WIQ in the participants based on the previous studies [27–29] and its validation was well evaluated in Chinese diabetic adults [30]. In order to further assess health-related quality of life during the clinical intervention process, we used the EuroQol-5 Dimensions questionnaire (EQ-5D) and SF-12. EQ-5D tracked the health status with 5 dimensions – mobility, self-care, usual activities, pain/discomfort, and anxiety/depression – and there were 3 levels in each dimension. The SF-12 questionnaire was designed to perform the physical components summary (PCS) and mental health component summary (MCS). The translated version of EQ-5D and SF-12 were recently recommended in the analysis of quality of life among Asians with diabetes [31–35].

Secondary outcomes were: (1) anthropometric measurement of height and weight for body mass index (BMI) and waist circumference; (2) blood assays of fasting glucose level, insulin, HbA1c level, total serum glyceride (TG), and serum LDL-cholesterol; and (3) inflammatory factors of serum C-reactive protein (CRP), inflammatory cytokines (tumor necrosis factor (TNF)- α and interleukin (IL)-6), plasma lipid peroxidation mediators (malondialdehyde (MDA)), and stress oxidation factors superoxide dismutases (SOD), and glutathione (GSH). In brief, fasting plasma glucose level was measured using EKF's Biosen analyser (Boerne, Tx, USA). Insulin was measured through electro-chemiluminescence immunoassay with the Elecsys[®] analyzer (Roche Diagnostics, Mannheim, Germany). HbA1c level was measured using direct enzymatic assay (Diazyme Laboratories, CA, USA). TG and LDL-cholesterol were analyzed by using the Hitachi 717 Autoanalyzer (Roche Diagnostic GmbH, Germany). Serum CRP was measured by particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). The serum levels of IL-6 and TNF- α were measured using a high-sensitivity Luminex-based magnetic bead assay (Bio-Rad Inc, Hercules, California, USA) according to the manufacturer's protocols. For all measurements, an average of at least 2 readings was taken for value analyses. The level of MDA was determined by using the TBARs (thiobarbituric acid reactive substances) method; GSH activity was measured by using cumene hydroperoxide and 1-chloro-2, 4-dinitrobenzene as substrates; and SOD activity was determined through inhibition of the reaction of xanthine oxidation [12,15].

Sample size

Sample size estimates were based on the primary outcome measure, symptoms, and physical status. To detect a change of 25% in the ABPI and physical functioning scales between the 2 matched groups and based on an α level of 0.05 and 10% dropout rate, a sample size of 120 participants was required (60 in each group).

Statistical methods

In this study we examined the hypothesis that hydrotherapy would give significantly more therapeutic benefit than acupuncture alone. The effect size (ES, mean change between follow-up and baseline divided by the standard deviation of the group's baseline scores) was calculated for responsiveness in terms of measured outcomes with significant improvement between the intervention group and the control group. The values represent improvements (positive) or reduction (negative) in the number of standard deviations of the baseline scores (ES) and ES ≥ 0.80 represents large, ≥ 0.50 and ≤ 0.79 is considered as moderate, ≥ 0.20 and ≤ 0.49 as small, and ≤ 0.19 as very small. Continuous variables are reported as mean (SD) or median (interquartile range) and categorical variables as number (%). Between-group differences were assessed using the

t test, Wilcoxon test, χ^2 test, or Fisher exact test, as appropriated. The associations between the outcomes of intervention, (LVC, physical measurements, and self-reported evaluation) and these variables were tested by using logistic regression analysis.

Results

Patients and LEAD features

In the current study, we screened 193 diabetic patients between November 2015 and March 2016. Of these, 39 did not meet the inclusion criteria mainly because of severe renal dysfunction, ventricular failure, and surgical reasons, as well as having received complementary or/and physical therapies in the past 3 months. About 10% (n=19) were not interested, were unwilling to participate in a randomized trial, or were not available during the intervention period. A total of 135 were enrolled and 9 patients refused to provide written informed consent. One hundred twenty-six were randomized in the rehabilitation center for eligibility. For diabetic duration, LEAD severity classified by ABPI degrees, and ECQ grades, the morbidities and other risk factors were not significantly different between the age-matched groups. Management of empirical treatment did not differ significantly between the intervention and the control groups at baseline. Description of recruited participants is shown in Table 2. As the intervention progressed, 5 patients (2 in the intervention group and 3 in the control group) withdrew because of unwillingness to participate, transportation problems, and family commitments. The final post-intervention evaluation and 6-week follow-up were completed by 121 patients.

Primary outcomes

Post-intervention significantly reduced the severity of intermittent claudication symptoms in the intervention group of acupuncture plus thermal hydrotherapy. First, the magnitude of intervention was the changed level of ABPI. In one side, 15-week intervention and 6-week follow-up, as compared to the baseline level, resulted in an increased level of ABPI from 0.78 to 0.82 and 0.81; On the other side, the control group decreased the level from 0.74 to 0.73 (Table 3). The results were evaluated for a standardized effect size (ES) showing that the combined therapy caused in an overall improvement of ABPI degrees compared with the control (Table 4). There were three participants (4.60%) showing a normal ABPI after treatment and follow-up, and four participants (6.45%) in the control group progressing to a moderate degree in the observation period. Secondly, upon the ECQ evaluation score, the intervention group demonstrated significant improvements with decreased proportion of moderate/severe claudication with rest pain (Grade 2) by 7.40% (95%, -0.11 to -13.7; ES,

Table 2. Baseline characteristics of study groups.

| Characteristics | Intervention (N=64) | | Control (N=62) | |
|-----------------------------------|---------------------|--------|----------------|--------|
| Age (yrs.) | 60.4 | (8.3) | 60.1 | (8.9) |
| Female (%) | 49 | | 54 | |
| Duration of diabetes (years) | 9.8 | (1.9) | 9.4 | (2.0) |
| Diabetic measures | | | | |
| Fasting glucose (mg/dl) | 176.5 | (20.7) | 162.3 | (21.2) |
| Insulin (μ U/ml) | 42.8 | (7.9) | 45.8 | (8.2) |
| ABPI | 0.78 | (0.15) | 0.74 | (0.17) |
| Duration of LEAD (years) | 1.1 | (0.2) | 0.9 | (0.2) |
| Diabetic treatment | | | | |
| Oral hypoglycemic drugs (%) | 11.9 | | 9.4 | |
| Oral hypoglycemic and insulin (%) | 88.1 | | 90.6 | |
| Anthropometric measures | | | | |
| Waist circumference (cm) | 95.6 | (12.3) | 98.5 | (11.9) |
| Waist-to-Hip Ratio | 0.75 | (0.81) | 0.74 | (0.87) |
| BMI | 30.8 | (4.1) | 31.2 | (4.2) |
| Physical measure | | | | |
| Lower-body flexibility | -4.91 | (0.45) | -4.64 | (0.38) |
| Hip joint flexibility | 79.6 | (8.1) | 83.7 | (8.6) |
| Lower-body strength and endurance | 12.9 | (1.4) | 13.1 | (1.7) |
| Medication history | | | | |
| β -blockers | 88.5 | | 89.1 | |
| RAS blockers* | 91.5 | | 93.9 | |
| Calcium channel blockers | 56.3 | | 49.7 | |
| Antifibrinolytic use | 56.1 | | 55.8 | |
| Lipid-modifying agent | 59.1 | | 61.2 | |
| Other risk factors (%) | | | | |
| Smoking | | | | |
| Current | 2.3 | | 1.9 | |
| Previous | 54.2 | | 60.3 | |
| Never | 43.5 | | 37.8 | |
| Alcohol drinking | | | | |
| Current | 1.1 | | 1.0 | |
| previous | 59.9 | | 60.5 | |
| Never | 39.0 | | 38.5 | |
| Hypertension | 59.9 | | 57.2 | |

Table 2. Baseline characteristics of study groups.

| Characteristics | Intervention (N=64) | Control (N=62) |
|--------------------------------|---------------------|----------------|
| Hyperlipidemia | | |
| Serum TG | 45.6 | 41.8 |
| Serum LDL-C | 56.9 | 60.7 |
| Arthritis in lower extremity | 3.7 | 2.9 |
| Respiratory disease | 15.9 | 19.1 |
| Renal dysfunction [#] | 25.7 | 31.6 |
| Coronary artery disease | 42.9 | 41.5 |
| Hepatic disease | 3.1 | 3.4 |

Acupuncture plus hydrotherapy; Data are means (SD) or n (% percentage); ABPI – ankle-brachial pressure index; * represents angiotensin-converting enzyme inhibitors and angiotensin receptor blockers; TG – total glyceride, LDL-C – low-density lipoprotein cholesterol; [#] glomerular filtration rate (GFR) ≤ 60 ml/min/1.73 m².

Table 3. The main outcomes in baseline, post-15-week acupuncture plus hydrotherapy, and 6-week follow-up periods.

| Parameter | Acupuncture+Hydrotherapy | | | | Control | | | |
|---|--------------------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|------|
| | Baseline | Intervention | Follow-up | P [#] | Baseline | Intervention | Follow-up | P |
| Biochemistry measure | | | | | | | | |
| Fasting glucose (mg/dl) | 176.5 (20.7) | 170.1 (18.2) | 170.9 (19.8) | 0.31 | 162.3 (21.2) | 160.1 (20.7) | 161.7 (22.9) | 0.31 |
| Insulin (uIU/ml) | 42.8 (7.9) | 40.4 (6.9) | 42.0 (7.8) | 0.27 | 45.8 (8.2) | 40.1 (8.9) | 40.7 (6.7) | 0.24 |
| HbA1C | 6.8 (1.3) | 6.9 (1.6) | 6.9 (1.8) | 0.31 | 7.0 (1.4) | 6.7 (1.4) | 6.7 (1.6) | 0.21 |
| C-reactive protein (mg/dl) | 15.2 (3.3) | 15.9 (3.2) | 15.7 (3.5) | 0.29 | 16.6 (3.5) | 15.9 (3.2) | 16.9 (3.4) | 0.32 |
| Interleukin (IL)-6 (pg/ml) | 1.39 (0.19) | 1.32 (0.17) | 1.27 (0.20) | 0.11 | 1.32 (0.22) | 1.39 (0.29) | 1.37 (0.24) | 0.21 |
| (TNF)- α (pg/ml) | 0.66 (0.05) | 0.60 (0.06) | 0.62 (0.05) | 0.14 | 0.63 (0.07) | 0.67 (0.05) | 0.69 (0.07) | 0.17 |
| MDA (μ mol/l) | 2.79 (0.81) | 2.33 (0.73) | 2.30 (0.74) | 0.10 | 2.85 (0.80) | 2.84 (0.81) | 2.81 (0.77) | 0.13 |
| SOD (U/ml) | 1.75 (0.37) | 1.59 (0.38) | 1.60 (0.39) | 0.09 | 1.79 (0.32) | 1.78 (0.35) | 1.80 (0.33) | 0.17 |
| GSH (μ mol/l) | 1.92 (0.46) | 2.07 (0.50) | 2.19 (0.44) | 0.09 | 2.00 (0.42) | 1.98 (0.36) | 2.02 (0.35) | 0.16 |
| Hemodynamics | | | | | | | | |
| ABPI | 0.78 (0.15) | 0.82 (0.19) | 0.81 (0.19) | 0.06 | 0.74 (0.17) | 0.73 (0.18) | 0.73 (0.20) | 0.09 |
| Leg vascular conductance (ml/min/mm Hg) | 3.03 (4.2) | 3.64 (4.2) | 3.67 (4.1) | <0.05 | 3.02 (4.3) | 3.31 (4.5) | 3.10 (4.6) | 0.07 |
| Blood pressure (mmHg) | | | | | | | | |
| Systolic | 159.3 (17.3) | 161.4 (18.2) | 160.8 (17.1) | 0.09 | 161.2 (17.8) | 160.7 (17.7) | 161.9 (18.1) | 0.10 |

Table 3 continued. The main outcomes in baseline, post-15-week acupuncture plus hydrotherapy, and 6-week follow-up periods.

| Parameter | Acupuncture+Hydrotherapy | | | | Control | | | P |
|--|--------------------------|-------------------------|-------------------------|-------|-------------------------|-------------------------|-------------------------|------|
| | Baseline | intervention | Follow-up | P# | Baseline | intervention | Follow-up | |
| Diastolic | 91.9 (10.3) | 92.0 (10.9) | 92.4 (11.1) | 0.09 | 92.2 (10.1) | 92.6 (11.7) | 93.7 (10.9) | 0.09 |
| Physical status | | | | | | | | |
| Lower-body flexibility, median (IQR) | | | | | | | | |
| Chair-sit-and-reach | -5.99 (-3.51, -9.96) | -5.35 (-3.43, -9.37) | -5.82 (-3.89, -9.11) | 0.09 | -5.47 (-3.25, -8.79) | -5.49 (-3.30, -9.01) | -5.16 (-3.09, -8.19) | 0.15 |
| Hip joint flexibility | 78.9 (46.3, 128.8) | 80.9 (49.7, 134.6) | 83.7 (48.8, 134.9) | 0.07 | 79.8 (48.5, 132.8) | 79.9 (48.1, 146.3) | 77.9 (46.8, 140.9) | 0.21 |
| Strength/endurance | 10.5 (6.2, 19.1) | 11.9 (7.2, 21.8) | 12.7 (8.2, 23.5) | 0.04 | 11.0 (6.6, 19.6) | 10.9 (6.5, 18.4) | 10.9 (6.6, 19.1) | 0.41 |
| Aerobic walking endurance | | | | | | | | |
| 4-M walking speed (m/sec) | 0.79 (0.11) | 0.84 (0.12) | 0.82 (0.15) | 0.07 | 0.81 (0.12) | 0.80 (0.14) | 0.79 (0.14) | 0.18 |
| Self-reported measures | | | | | | | | |
| WIQ distance score (0-100 scale) | 30.1 (18.2, 54.2) | 32.8 (20.3, 54.4) | 32.3 (22.2, 51.5) | 0.04 | 31.7 (19.2, 54.9) | 32.2 (19.6, 54.1) | 31.9 (18.6, 54.7) | 0.11 |
| WIQ speed score (0-100 scale) | 34.7 (20.2, 54.6) | 34.9 (21.1, 61.3) | 34.9 (22.2, 61.0) | 0.08 | 33.8 (19.8, 59.6) | 33.8 (19.4, 59.3) | 33.5 (19.0, 58.8) | 0.18 |
| EQ-5D dimension problems (%) | 21.4 | 22.2 | 21.9 | 0.06 | 22.0 | 21.5 | 21.9 | 0.11 |
| EQ-5D index (0-1) score, median (IQR) | 0.57 (0.35, 1.12) | 0.64 (0.38, 1.17) | 0.69 (0.41, 1.28) | 0.04 | 0.57 (0.34, 1.04) | 0.54 (0.31, 0.99) | 0.53 (0.30, 0.97) | 0.11 |
| SF-12, media (IQR) | | | | | | | | |
| PCS | 30.7 (18.6, 54.5) | 35.8 (23.9, 72.1) | 36.3 (24.2, 73.3) | 0.03 | 31.3 (18.7, 55.3) | 30.9 (19.9, 55.1) | 32.9 (19.8, 56.8) | 0.27 |
| MCS | 42.8 (25.6, 74.8) | 49.7 (29.8, 90.4) | 51.7 (31.4, 93.8) | <0.05 | 42.1 (25.2, 75.5) | 43.2 (25.9, 75.8) | 43.7 (26.2, 78.9) | 0.07 |

Data are means (SD) or n (%), percentage) or interquartile range (IQR); # a linear trend value. TNF – tumor necrosis factor; MDA – malondialdehyde; SOD – superoxide dismutase; GSH – reduced glutathione; WIQ – the walking impairment questionnaire; PCS – the physical component summary score; MCS – mental health component summary score.

0.55, $P < 0.02$), thus occurring a left shift with increased portion of Grade 1 (mild intermittent claudication) relative to the control group (Table 4). The benefit effect on the prevalence of intermittent claudication based upon ECQ was remained at the end of 6-week follow-up (Table 4). Thirdly, we measured the leg vascular conductance to evaluate vascular action. The average of LVC at the basal stage was lower than the values of previous reports in the elderly non-diabetic and insulin-resistant subjects [37]. There was overall statistical change of leg vascular conductance only in acupuncture plus hydrotherapy group through baseline to the follow-up point ($P < 0.05$) (Table 3); As compared with the control, 15-week combined therapy resulted in increases of 0.32 ml/min/mmHg (95% CI,

0.08 to 0.56; ES, 0.43, $P < 0.02$) at the end point of post-intervention and 0.56 (ml/min/mmHg (95% CI, 0.13 to 0.99; ES, 0.59, $P < 0.01$) at the 6-week follow-up (Table 4). Fourthly, no significant change in blood pressure was found on all of these outcomes between baseline, and post-intervention / follow-up points in both groups.

Finally, across the 15-week's intervention and 6-week follow-up, the physical status was evident changed in lower body flexibility and aerobic walking endurance (Table 3). The significant difference between the intervention group and the control were found in some physical functions represented by the chair-sit and reach, strength/endurance and 6-min

Table 4. Results of variables post-intervention group and follow-up vs. control group.

| Outcome | 15-week intervention vs. control | | | 6-week Follow-up vs. control | | |
|-------------------------------|----------------------------------|-------------------|-------|------------------------------|-------------------|-------|
| | Difference (CI 95%) | ES (CI 95%) | p | Difference (CI 95%) | ES (CI 95%) | p |
| Biochemistry measures | | | | | | |
| C-reactive protein | 1.40 (0.3, 2.6) | 0.31 (0.26, 0.36) | 0.07 | 0.20 (-0.09, 3.5) | 0.11 (0.06, 0.16) | 0.15 |
| IL-6 | -0.14 (-0.03, -0.26) | 0.32 (0.28, 0.36) | 0.06 | -0.17 (-0.09, -0.28) | 0.50 (0.45, 0.55) | <0.05 |
| TNF-α | -0.13 (-0.06, -0.21) | 0.52 (0.46, 0.59) | <0.05 | -0.13 (-0.06, -0.21) | 0.52 (0.46, 0.59) | <0.05 |
| MDA | -0.45 (-0.21, -0.69) | 0.44 (0.29, 0.59) | <0.05 | -0.45 (-0.22, -0.69) | 0.41 (0.30, 0.53) | <0.05 |
| SOD | -0.15 (-0.06, -0.24) | 0.35 (0.29, 0.41) | 0.05 | -0.16 (-0.05, -0.27) | 0.38 (0.26, 0.51) | <0.05 |
| GSH | 0.13 (0.4, 0.23) | 0.27 (0.11, 0.42) | 0.09 | 0.25 (0.12, 0.38) | 0.43 (0.17, 0.69) | <0.05 |
| Hemodynamics | | | | | | |
| ABPI | 0.05 (0.01, 0.09) | 0.38 (0.10, 0.66) | <0.05 | 0.04 (0.008, 0.072) | 0.32 (0.09, 0.55) | <0.05 |
| ECQ (%) | | | | | | |
| Grade 1 | 7.5 (1.2, 13.8) | 0.54 (0.10, 0.98) | <0.05 | 9.0 (1.5, 16.5) | 0.49 (0.14, 0.84) | <0.02 |
| Grade 2 | -7.4 (-1.1, -13.7) | 0.55 (0.10, 1.0) | <0.02 | -9.0 (-1.4, -16.6) | 0.58 (0.14, 1.02) | <0.02 |
| Leg vascular conductance | 0.32 (0.08, 0.56) | 0.43 (0.14, 0.72) | <0.02 | 0.56 (0.13, 0.99) | 0.59 (0.19, 0.99) | <0.01 |
| Blood pressure (mmHg) | | | | | | |
| Systolic | 2.60 (0.9, 4.3) | 0.28 (0.04, 0.52) | 0.15 | 0.80 (0.2, 1.4) | 0.10 (0.02, 0.18) | 0.25 |
| Diastolic | -0.3 (0.09, -0.51) | 0.12 (0.01, 0.23) | 0.24 | -1.0 (0.25, -1.75) | 0.09 (0.01, 0.17) | 0.29 |
| Physical status | | | | | | |
| Chair-sit-and-reach | 1.08 (0.9, 2.08) | 0.53 (0.22, 0.84) | <0.02 | 0.88 (0.2, 1.59) | 0.49 (0.22, 0.74) | <0.05 |
| Hip joint flexibility | 3.9 (0.3, 7.6) | 0.19 (0.12-0.27) | 0.10 | 17.1 (1.4, 33.2) | 0.51 (0.45-0.57) | <0.05 |
| Strength/endurance | 2.8 (1.1, 4.8) | 0.52 (0.27, 0.77) | <0.02 | 3.5 (1.3, 5.9) | 0.55 (0.36, 0.74) | <0.01 |
| 4-M walking speed | 0.06 (-0.7, 0.11) | 0.38 (0.32, 0.44) | 0.06 | 0.05 (-0.8, 0.10) | 0.20 (0.15, 0.25) | 0.08 |
| Self-reported measures | | | | | | |
| WIQ distance score | 7.7 (0.7, 14.8) | 0.53 (0.46, 0.60) | <0.05 | 11.0 (1.3-21.9) | 0.65 (0.59, 0.71) | <0.02 |
| WIQ speed score | 4.7 (0.6, 9.1) | 0.50 (0.42, 0.58) | <0.05 | 4.7 (0.6-9.1) | 0.50 (0.42, 0.58) | <0.05 |
| EQ-5D index score | 0.10 (0.02, 0.19) | 0.50 (0.43-0.57) | <0.05 | 0.16 (0.04, 0.31) | 0.52 (0.46-0.58) | <0.05 |
| SF-12 | | | | | | |
| PCS | 5.5 (1.2, 9.8) | 0.57 (0.74, 0.94) | 0.01 | 4.0 (0.5, 7.5) | 0.51 (0.22, 0.80) | <0.05 |
| MCS | 6.0 (0.2, 11.8) | 0.22 (0.17, 0.27) | 0.08 | 7.5 (0.3, 15.5) | 0.33 (0.27, 0.39) | 0.06 |

% – percentage; IL – interleukin; TNF – tumor necrosis factor; ES – effect size; additional definitions refer to Table 3.

walk distance assessments. Similarly, the effect remained after 6-week follow-up (Table 4).

Secondary outcomes

There were no significant between-group differences in fasting blood glucose level, Hb1Ac, and insulin levels across the intervention and follow-up period, nor were there any significant change in blood pressure and anthropometric values between groups (Tables 3, 4).

Serum inflammatory analyses were performed comparing responses in the intervention and control groups. No significant effect of acupuncture plus hydrotherapy was found on inflammatory factor CRP, IL-6, SOD, and GSH levels. However, the levels of TNF-α and MDA were down-regulated after the therapy. At the endpoint of 6-week follow-up, a significant decline in serum levels of IL-6, TNF-α, MDA, and SOD and an increase in GSH level were observed (Tables 3, 4).

The combined therapy significantly improved the results of the self-reported measures of walking impairment questionnaire

Table 5. Association of TNF- α , MDA, SOD, and GSH levels with outcomes in the acupuncture plus hydrotherapy intervention and follow-up in diabetics with mild LEAD.

| Outcome [#] | TNF- α | | | MDA | | | SOD | | | GSH | | |
|----------------------|---------------|-----------|-------|------|-----------|-------|------|-----------|-------|------|-----------|-------|
| | OR | 95% CI | P | OR | 95% CI | P | OR | 95% CI | P | OR | 95% CI | P |
| LVC | 0.58 | 0.42–0.78 | 0.02 | 0.69 | 0.52–0.93 | 0.01 | 0.74 | 0.57–0.96 | 0.01 | 1.28 | 1.03–1.64 | 0.005 |
| Chair-sit-and-reach | 0.54 | 0.41–0.75 | 0.005 | 0.61 | 0.48–0.84 | 0.008 | 0.72 | 0.66–0.90 | 0.009 | 1.22 | 1.05–1.69 | 0.009 |
| Strength/endurance | 0.49 | 0.44–0.70 | 0.004 | 0.55 | 0.43–0.69 | 0.004 | 0.76 | 0.69–0.89 | 0.02 | 1.21 | 1.06–1.69 | 0.011 |
| WIQ | 0.56 | 0.43–0.69 | 0.009 | 0.66 | 0.56–0.79 | 0.01 | 0.81 | 0.73–0.96 | 0.04 | 1.27 | 1.03–1.66 | 0.004 |
| PCS | 0.51 | 0.40–0.66 | 0.001 | 0.61 | 0.45–0.81 | 0.007 | 0.88 | 0.71–0.99 | 0.04 | 1.22 | 1.06–1.62 | 0.008 |

[#] Adjusted for age, sex, Treatment, medical history and other factors. OR – odds ratio; CI – confidence interval; LVC – leg vascular conductance. Additional definitions refer to Table 3.

and SF-12 subscale-PCS across the intervention period. The analysis of health-related quality of life was evident in better response in the intervention in all the EQ-5D dimensions and index scores and SF-12 MCS score (Tables 3, 4).

After adjusting for age, sex, medical history, and other risk factors, the ORs (odd ratios) for having a reduced TNF- α level were 0.58, 0.54, 0.49, 0.56, and 0.51 for LVC, chair-sit-and-reach, strength and endurance, WIQ, and PCS, respectively, in the intervention group. The logistic regression analysis showed the significant association of outcomes with intervention therapy ($P=0.02$, 0.005, 0.009, and 0.001 respectively) (Table 5). Similarly, MDA and SOD levels were positively associated with these outcome variables in the regression analysis in the overall intervention and follow-up (Table 5). Negative correlation trends were observed for the associations of GSH level with LVC, physical measurement, and self-reported evaluation.

Tolerance and adverse events

Across the period of trial, the duration and frequency of acupuncture were acceptable and hot spring thermal therapy was well tolerated when following the instructions of the study protocol (see Methods). No unexpected serious adverse events took place during or after the intervention. There were 8 occurrences of soreness and mild bruises around the acupoint site in 5 patients and 3 reports of a mild dermal sensitivity in 3 patients, which were treated as outpatient at the hospital; adverse effects were not clinically concerning.

Discussion

This study shows that the intervention can be applied to diabetic patients with symptomatic LEAD for 20 weeks. This approach is successful in real-world practice when combining

acupuncture with thermal hydrotherapy among the participants who had an average 7.9 years of diabetes and 2.1 years of LEAD with mild symptomatic manifestation. Traditional acupuncture therapy works by enhancing circulation in the lower extremities and reducing pain/numbness. To the best of our knowledge, the present study is the first to evaluate the effect of the combination of acupuncture and hydrotherapy in comparable samples of diabetic patients with symptomatic LEAD, as it enables us to determine whether the intervention will supply specific information about safety and benefits. Our study aimed to develop an approach targeting reduction of body load and avoidance of high-impact activity in maintenance and improvement of leg function in subjects with LEAD. During the intervention, the subjects reported that post-acupuncture therapy in the morning gave them a sense of pleasant calmness or/and relaxation, and aquatic movement in the afternoon altered overload of legs by transferring load from affected areas to other areas. The intensity and frequency for 15 weeks in the current protocol in the combined acupuncture with hydrotherapy did not increase the risk of skin bruise, ulceration, or development of Charcot joint, which can occur in weight-bearing exercise [36]. A previous report showed the concern of a risk of declined ventricular function during warm water immersion among the people with systolic heart failure [37]. As compared with aerobic exercise, which may elicit beneficial effects in endothelial function [38,39], hydrotherapy in this study did not require more specific skills and was more easily adjusted in intensity for safe continuation among diabetic patients with mild LEAD. In clinical practice, it is less likely than aerobic exercise to cause a host of undesirable and potentially adverse effects, including injuries and foot trauma. We assessed blood pressure, heart rate, and skin response to evaluate the tolerance of subjects to acupuncture plus hydrotherapy. There were no significant or serious adverse events. Although the 2 therapies may induce nerve damage and dermal sensitization/irritation due to needle penetration and the

potential dissolved materials, the occurrence of adverse nerve damage and skin response, and events requiring immediate withdrawal had no significant effect on data collection, power analysis, or data validity.

Our findings show that the benefit of the intervention was detected with post-intervention decreases in serum TNF- α and MDA levels in the subjects compared with the controls. Furthermore, the changes in clearing free radicals were inversely correlated with alternations in the leg vascular conductance, chair-sit-and-reach, strength/endurance, WIQ, and PCS self-reported evaluations. These data indicate that the combined complementary therapies, as compared with the control, demonstrated measurable improvements in inflammatory homeostasis. Although the mechanisms through which the diabetic condition deteriorates the distal hemodynamic homeostasis and leads to arterials dysfunction are not fully elucidated, the roles of accumulated glycosylation end-products and arterial inflammation has been documented [40]. Recent studies showed that chronic TNF- α elevation impairs glucose uptake and induces insulin resistance and fat oxidation and stimulates IL-6 and CRP production. MDA, an indicator of lipid peroxidation and oxidative stress *in vivo*, is elevated in diabetic patients with macroangiopathy [41] and is an independent predictor in diabetics with PAD [42]. The effects of acupuncture plus hydrotherapy on TNF- α and MDA associated with diabetic mild LEAD are yet to be fully understood. It is consistent with previous studies that acupuncture downregulates serum TNF- α in the rat model of COPD [43] and MDA in the rat model of hyperlipidemia [44]. Among people with bronchial asthma [45] and osteoarthritis [46], stimulation by thermal hydrotherapy is associated with immune suppression. Endothelial cells within the vessel wall of lower extremities in patients with LEAD may produce cytokines and oxygen-derived free radicals due to hypoxia and reoxygenation [47]. Furthermore, TNF- α induces NF- κ B activation and inflammatory gene expression in atherogenic and inflammatory process [48] and regulates α atrial natriuretic polypeptide (α -ANP) and vasopressin. Thus, our findings suggest that the effect of acupuncture plus hydrotherapy can improve of the endothelial cells' anti-inflammatory property, reduction of free radicals, and regulate the hypothalamic-pituitary-adrenal axis function [49,50]. The other explanation is likely that an increase in serum antioxidant capacity, represented by total reactive antioxidant potentials (TRAP), would have been detected.

CRP, an inflammatory factor involved in endothelial dysfunction, can predict the high risk of progression to diabetes [51] and possibly the severity of cardiovascular comorbidity [52]. Population studies showed inflammatory markers such as CRP and IL-6 are negatively associated with beneficial effects of regular physical activity [53]. A recent study showed that CRP is associated with physical activity and risk of metabolic

syndrome among Chinese people [54]. The levels of CRP in our study were 15.2 mg/dl in the intervention group and 16.6 mg/dl in the control group, which is slightly higher than that in people 50–70 years old. However, no significant change in levels of CRP was found after intervention activity for 15 weeks and 6-week follow-up compared with the control therapy. This result is consistent with a recent study of acupuncture in diabetes [55], which may reflect different impacts on particular inflammatory markers due to therapy type, intensity, or duration.

Another of our findings was decreases in serum IL-6 and SOD levels and an increase in GSH at 6-week follow-up. Some studies showed an inverse correlation between systemic SOD activity and indices of glycemic control in diabetic patients [56]. However, our study does not allow conclusions to be drawn because there was no significant improvement in glycemic control. This may reflect differences in intervention methodology or study design. Type 2 diabetic patients have decreased GSH levels in different phases of the disease. There is an inverse correlation between GSH levels and the presence of DM complications [57]. Our findings suggest the intervention corrected the impaired GSH levels. Overall, our findings indicate that acupuncture plus hydrotherapy can improve leg conductance and physical function by alleviating oxidative stress and inflammation.

This suggests that the combination of acupuncture with hydrotherapy does bring closer the desired goal of reducing dyslipidemia and inflammation and stabilizing oxidative factors homeostasis in patients with lower-limb diabetic macroangiopathy. Clinical treatment generally includes percutaneous balloon angioplasty, endarterectomy, radiological, pharmacological interventions, and amputation, while combined treatment improved diabetic LEAD, which is a relentlessly serious condition, especially considering the high rate of amputation and mortality [58]. To better understand the possible mechanisms underlying the protective effect of complementary therapy in lower-extremity circulation, it would be interesting to investigate levels of uncoupling proteins (UCPs), such as anion carriers in the mitochondrial inner membrane, which inhibit free radical generation, and levels of endothelial-derived nitric oxide [59] and reactive oxygen species (ROS) [60].

We assessed symptoms and walking performance among the patients with mild and moderate LEAD at the end of the combined therapy and showed a reduction in the difficult barriers of objective measures, especially in physical capability and improved hemodynamic outcomes in ABPI and ECQ evaluation. The benefits were also associated with composite outcomes in self-reported measures in which WIQ distance score and speed score were greater and relevant general health quality levels represented by EQ-5D and PCS were significantly improved after the intervention. Subjects with LEAD had lower

LEAD-related physical measure (WIQ) and lower QOL scores (EQ-5D and SF-12) at baseline. After 15-week intervention of acupuncture plus hydrotherapy, a significant improvement from baseline was observed in WIQ distance (ES, 0.53, CI, 0.46–0.60, $P < 0.05$) and WIQ speed (ES, 0.50, CI, 0.42–0.58, $P < 0.05$), and QOL scores (Table 4) compared with the control group. Our results suggest the intervention significantly improved certain LEAD-related symptoms measures and general health-related quality of life. It supported the previous studies which showed improved sleep quality, physical function/symptoms, and psychological disorders in patients with fibromyalgia by using acupuncture [61] or hydrotherapy [62]. The benefits were sustained at the end of 6-week follow-up.

The 15-week intervention and 6-week follow-up statistically increased leg vascular conductance (LVC) and showed positive associations between LVC and low extremity strength/endurance, indicating the reduction in partial sympathetic vasoconstriction [63] and restoration of blood flow [64]. As a result, the muscle chemoreflex may functionally impact the blood pressure response [64] to certain physical activity such as sit-and-reach. However, our findings showed no significant change in body weight, HbA1c, or glycemic control. A recent study showed that aerobic training (3.4 times per week for 18 weeks) reduced HbA1c by 0.6% and decreased risk of diabetic-related complications by 32% compared to the control groups [65]. The result likely in part reflects the therapeutic difference between complimentary therapies such as acupuncture and hydrotherapy and other aerobic training in delivering biosystemic rejuvenation.

Limitations

This investigation had a sound complementary study design and adequate power to examine the hypothesis in randomized

control analysis. However, our study may have limited relevance to socioeconomic position, which could affect diabetes progress, especially for women [46]. Second, the study was unable to account for subject's genotype with polymorphism of cytokines' regulating expression and plasma clearance. Third, only a control group was used to compare with intervention therapy in this study, and the evaluation should include comparison with acupuncture alone or hydrotherapy alone. Fourth, we did not perform long-term assessment of effects of the intervention.

Conclusions

In the elderly diabetic type 2 patients with LEAD, 15-week acupuncture plus hydrotherapy compared with the control was safe and significantly prevented LEAD progression. The intervention without significant glycemic-controlling effects exerts anti-inflammatory effects and improves physical function in type 2 diabetic patients with mild LEAD. The mitigation in inflammatory cytokines is likely associated with improvements in hemodynamics, physical measures, and self-reported measures. This study suggests that acupuncture plus hydrotherapy may be useful in the treatment of diabetic patients with mild LEAD.

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Conflict of interest

None.

References:

1. Abi Khalil C, Roussel R, Mohammedi K et al: Cause-specific mortality in diabetes: Recent changes in trend mortality. *Eur J Prev Cardiol*, 2012; 19: 374–81
2. van Dieren S, Beulens JW, van der Schouw YT et al: The global burden of diabetes and its complications: An emerging pandemic. *Eur J Cardiovasc Prev Rehabil*, 2010; 17(Suppl. 1): S3–8
3. Al-Qubaissi, Khamis Y, Peter A, Yohannes AM. The effectiveness of hydrotherapy in the management of rheumatoid arthritis: A systematic review. *Musculoskeletal Care*, 2013; 11: 3–18
4. Fransen M, Lillias N, Julie W et al: Physical activity for osteoarthritis management: A randomized controlled clinical trial evaluating hydrotherapy or Tai Chi classes. *Arthritis Care Res*, 2007; 57: 407–14
5. Castro-Sánchez, Adelaida M, Guillermo A et al: Hydrotherapy for the treatment of pain in people with multiple sclerosis: A randomized controlled trial. *Evid Based Complement Alternat Med*, 2012; 2012: 473963
6. Cooper K, Philip H, Clare R, Kate T: Prevalence of visits to five types of complementary and alternative medicine practitioners by the general population: A systematic review. *Complement Ther Clin Pract*, 2013; 19: 214–20
7. Das UN: Is obesity an inflammatory condition? *Nutrition*, 2001; 17: 953–66
8. Fernandez-Real JM, Ricart W: Insulin resistance and chronic cardiovascular in Xammatory syndrome. *Endocr Rev*, 2003; 24: 278–301
9. Pradhan AD, Manson JE, Rifai N et al: C-reactive protein, interleukin-6 and risk of developing type 2 diabetes mellitus. *JAMA*, 2001; 286: 327–34
10. Ridker PM, Buring JE, Cook NR, Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: An 8-year follow-up of 14719 initially healthy American women. *Circulation*, 2003; 107: 391–97
11. Tzoulaki I, Murray GD, Lee AJ et al: C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh artery study. *Circulation*, 2005; 112: 967–83
12. Madamanchi NR, Vendrov A, Runge MS: Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol*, 2005; 25: 29–38
13. Baynes JW, Thorpe SR: Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes*, 1999; 8: 1–9
14. Förstermann U: Oxidative stress in vascular disease: Causes, defense mechanisms and potential therapies. *Nat Clin Pract Cardiovasc Med*, 2008; 5: 338–49
15. Miller FJ, Gutterman DD, Rios CD et al: Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. *Circulation Res*, 1998; 82: 1298–305

16. Bergmark C, Wu R, De Faire U et al: Patients with early-onset peripheral vascular disease have increased levels of autoantibodies against oxidized LDL. *Arterioscler Thromb Vasc Biol*, 1995; 15: 441-45
17. Al-Qaisi M, David MN, David HK, Sam K: Ankle brachial pressure index (ABPI): An update for practitioners. *Vasc Health Risk Manag*, 2009; 5: 833-41
18. Shen Q, Jia W, Bao Y et al: [Prevalence of peripheral vascular disease in diabetics and impaired glucose regulation subjects: A community-based study.] *Zhonghua Yi Xue Za Zhi*, 2006; 86: 1530-33 [in Chinese]
19. Bennett P, Gregory L, Stanley S et al: Validation of the Edinburgh claudication questionnaire in 1st generation black African-Caribbean and South Asian UK migrants: A sub-study to the ethnic-echocardiographic heart of England screening (E-ECHOES) study. *BMC Med Res Methodol*, 2011; 11: 85
20. Lend GC, Fowkes FG: The Edinburgh Claudication Questionnaire: An improved version of the WHO/Rose Questionnaire for use in epidemiological surveys. *J Clin Epidemiol*, 1992; 45: 1101-9
21. MacAnaney O, Heather R, Donal O et al: Effect of type 2 diabetes on the dynamic response characteristics of leg vascular conductance during exercise. *Diab Vasc Dis Res*, 2011; 8: 12-21
22. Manson J, Michael R, Veronica J et al: Effect of tai chi on musculoskeletal health-related fitness and self-reported physical health changes in low income, multiple ethnicity mid to older adults. *BMC Geriatrics*, 2013; 13: 114
23. Takeshima N, Islam MM, Rogers ME et al: Effects of nordic walking compared to conventional walking and band-based resistance exercise on fitness in older adults. *J Sports Sci Med*, 2013; 12: 422-30
24. Masiero S, Patrizia P, Lara B et al: Supervised training and home-based rehabilitation in patients with stabilized ankylosing spondylitis on TNF inhibitor treatment: A controlled clinical trial with a 12-month follow-up. *Clin Rehabil*, 2013; 28: 562-72
25. Askew CD, Parmenter B, Leicht AS et al: Exercise & Sports Science Australia (ESSA) position statement on exercise prescription for patients with peripheral arterial disease and intermittent claudication. *J Sci Med Sport*, 2014; 17: 623-29
26. Brun JF, Bordenave J, Mercier A et al: Cost-sparing effect of twice-weekly targeted endurance training in type 2 diabetics: A one-year controlled randomized trial. *Diabetes Metab*, 2008; 34: 258-65
27. Ahimastos AA, Philip W, Christopher A et al: Effect of ramipril on walking times and quality of life among patients with peripheral artery disease and intermittent claudication: a randomized controlled trial ramipril among patients with PAD and claudication. *JAMA*, 2013; 309: 453-60
28. Jain A, Kiang L, Luigi F et al: The Walking Impairment Questionnaire stair-climbing score predicts mortality in men and women with peripheral arterial disease. *J Vasc Surg*, 2012; 55: 1662-73
29. Schiano V, Gregorio B, Giusy S et al: Functional status measured by walking impairment questionnaire and cardiovascular risk prediction in peripheral arterial disease: Results of the Peripheral Arteriopathy and Cardiovascular Events (PACE) study. *Vasc Med*, 2006; 11: 147-54
30. Jie W, Cui Y, Rong-Wen B et al: Validation of the Chinese version of the Walking Impairment Questionnaire in patients with both peripheral arterial disease and type 2 diabetes mellitus. *Diab Vasc Dis Res*, 2011; 8: 29-34
31. Wee H, Chee-Eng T, Su-Yen G, Shu-Chuen L: Usefulness of the Audit of Diabetes-Dependent Quality-of-Life (ADDQoL) questionnaire in patients with diabetes in a multi-ethnic Asian country. *Pharmacoeconomics*, 2006; 24: 673-82
32. Swee Sung S, Yen Goh S, Mong Bee Y et al: Audit of Diabetes-Dependent Quality of Life (ADDQoL) [Chinese version for Singapore] questionnaire. *Appl Health Econ Health Policy*, 2010; 8: 239-49
33. John W, Gandek B, Kosinski M et al: The equivalence of SF-36 summary health scores estimated using standard and country-specific algorithms in 10 countries: Results from the IQOLA project. *J Clin Epidemiol*, 1998; 51: 1167-70
34. Wong CK, Lo YY, Wong WH, Fung CS: The associations of body mass index with physical and mental aspects of health-related quality of life in Chinese patients with type 2 diabetes mellitus: Results from a cross-sectional survey. *Health Qual Life Outcomes*, 2013; 11: 142
35. Leung J, Timothy K, Dicken C et al: Visual functioning and quality of life among the older people in Hong Kong. *Int J Geriatr Psychiatry*, 2012; 27: 807-15
36. American Diabetes Association: Standards of medical care in diabetes – 2012. *Diabetes Care*, 2012; 35: 511-63
37. Sveälv BG, Täng MS, Cider A: Is hydrotherapy an appropriate form of exercise for elderly patients with biventricular systolic heart failure? *J Geriatr Cardiol*, 2012; 9: 408-10
38. Maiorana A, O'Driscoll G, Cheetham C et al: The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol*, 2001; 8: 860-66
39. Kim K, Valentine RJ, Shin Y, Gong K: Associations of visceral adiposity and exercise participation with C-reactive protein, insulin resistance, and endothelial dysfunction in Korean healthy adults. *Metabolism*, 2008; 7: 1181-89
40. Shashkin P, Nitin J, Yury M et al: Insulin and glucose play a role in foam cell formation and function. *Cardiovasc Diabetol*, 2006; 5: 13
41. Gallou G, Ruelland A, Legras B et al: Plasma malondialdehyde in type 1 and type 2 diabetic patients. *Clin Chim Acta*, 1993; 214: 227-34
42. Lapolla A, Piarulli F, Sartore G et al: Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. *Diabetes Care*, 2007; 30: 670-76
43. Li J, Wu S, Tang H et al: Long-term effects of acupuncture treatment on airway smooth muscle in a rat model of smoke-induced chronic obstructive pulmonary disease. *Acupunct Med*, 2016; 34: 107-13
44. Xie JP, Li W, Nong Y et al: Effects of electroacupuncture at Fenglong(ST40) on SOD and MDA in different organs of the hyperlipemia rat. *Chin Acupunct Moxibustion*, 2008; 28: 293-96
45. Fumihoro M, Yamaoka K, Hanamoto K et al: Elevation of antioxidant enzymes in the clinical effects of radon and thermal therapy for bronchial asthma. *J Radiat Res*, 2003; 44: 95-99
46. Yamaoka K, Fumihoro M, Katsumi H et al: Study on biologic effects of radon and thermal therapy on osteoarthritis. *J Pain*, 2004; 5: 20-25
47. Ala Y, Palluy J, Favero B et al: Hypoxia/reoxygenation stimulates endothelial cells to promote interleukin-1 and interleukin-6 production. Effects of free radical scavengers. *Agents Actions*, 1992; 37: 134-39
48. Csizsar A, Smith K, Labinsky N et al: Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: Role of NF-kappaB inhibition. *Am J Physiol Heart Circ Physiol*, 2006; 291: H1694-99
49. Mastorakos G, Weber JS, Magiakou MA et al: Hypothalamic-pituitary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin-6 in humans: Potential implications for the syndrome of inappropriate vasopressin secretion. *J Clin Endocrinol Metab*, 1994; 79: 934-39
50. Kageyama K, Watanobe H, Takebe K: *In vivo* evidence that arginine vasopressin is involved in the adrenocorticotropin response induced by Interleukin-6 but not by tumor necrosis factor- α in the rat. *Neuroimmunomodulation*, 1995; 2: 137-40
51. Kathryn T, Wat N, Tam S et al: C-reactive protein predicts the deterioration of glycemia in Chinese subjects with impaired glucose tolerance. *Diabetes Care*, 2003; 26: 2323-28
52. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW: C-reactive protein in healthy subjects associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*, 1999; 19: 972-78
53. Abramson JL, Vaccarino V: Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. *Arch Intern Med*, 2002; 62: 1286-92
54. Yu Z, Ye X, Wang J et al: Associations of physical activity with inflammatory factors, adipocytokines, and metabolic syndrome in middle-aged and older Chinese people. *Circulation*, 2009; 19: 2969-77
55. Firouzjaei A, Li G-C, Wang N et al: Comparative evaluation of the therapeutic effect of metformin monotherapy with metformin and acupuncture combined therapy on weight loss and insulin sensitivity in diabetic patients. *Nutr Diabetes*, 2016; 6: e209
56. Tho LL, Candlish JK, Thai AC: Correlates of diabetes markers with erythrocytic enzymes decomposing reactive oxygen species. *Ann Clin Biochem*, 1988; 25: 426-31
57. De Mattia G, Laurenti O, Bravi C et al: Effect of aldose reductase inhibition on glutathione redox status in erythrocytes of diabetic patients. *Metabolism*, 1994; 43: 965-68
58. Barzilay JJ, Kronmal RA, Bittner V et al: Coronary artery disease in diabetic patients with lower-extremity arterial disease: Disease characteristics and survival. A report from the Coronary Artery Surgery Study (CASS) registry. *Diabetes Care*, 1997; 20: 1381-87

59. Kobayashi MA, Watada H, Kawamori R, Maeda S: The role of uncoupling proteins in diabetes mellitus, analysis of inflammatory mediators in type 2 diabetes patients. *J. Diabetes Res*, 2013; 14: 315–24
60. Sharma A, Pascal B, Judy H: Targeting endothelial dysfunction in vascular complications associated with diabetes. *Int J Vasc Med*, 2012; 2012: 750126
61. Uğurlu FG, Sezer N, Aktekin L et al: The effects of acupuncture versus sham acupuncture in the treatment of fibromyalgia: A randomized controlled clinical trial. *Acta Reumatol Por*, 2017; 1: 32–37
62. Kyara S, Oliveira M, Tucano S et al: Effect of hydrotherapy on quality of life, functional capacity and sleep quality in patients with fibromyalgia. *Rev Bras Reumatol*, 2012; 52(6): 851–57
63. Sheriff DD, Rowell LB, Scher AM: Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am J Physiol Heart Circ Physiol*, 1993; 265: H1227–34
64. Scott M, Bell L, O'Hagan K, Clifford P: Muscle chemoreflex alters vascular conductance in nonischemic exercising skeletal muscle. *J Appl Physiol*, 1994; 77: 2761–66
65. UK Prospective Diabetes Study (UKPDS) Group: Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS-34). *Lancet*, 1998; 352: 854–65