Front. Med. 2025, 19(6): 1070−1085
https://doi.org/10.1007/s11684-025-1192-9

RESEARCH ARTICLE

A phase 1/2 pilot study of acupuncture and moxibustion at Zusanli (ST36) as a prophylactic treatment in middle-aged and elderly individuals

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Abstract **Zusanli (ST36), one of the most essential acupoints in clinical acupuncture practice, is considered as an intervention strategy for disease prevention and longevity promotion in traditional Chinese medicine (TCM). However, the prophylactic effects of ST36 have yet to be investigated in clinical studies. In the present study, we conducted a single-arm, open-label, pilot clinical trial to evaluate the prophylactic effects of stimulating the ST36 acupoint through acupuncture and moxibustion in middle-aged and elderly participants. Of the 85 participants who completed the first 2 months of acupuncture and moxibustion, 48 continued through the remaining 4 months to complete the full 6-month intervention. For 6 months acupuncture and moxibustion at ST36 significantly reduced levels of low-density lipoprotein (LDL) and uric acid (UA) in individuals with initially abnormal LDL and UA values. The median LDL levels decreased from 4.46 mmol/L (IQR 4.32–4.71) to 3.97 mmol/L (IQR 3.32–4.47) (P = 0.004) and the median UA levels decreased from 399.00 μmol/L (IQR 356.00–483.00) to 361.00 μmol/L (IQR 323.00–421.00) (P = 0.003). Additionally, ST36 intervention led to marked reductions in the levels of lactic dehydrogenase (LDH) (266.50 U/L (IQR 257.50–282.25) to 235.50 U/L (IQR 215.50–244.75), P < 0.001) at 6 months, and α-hydroxybutyrate dehydrogenase (α-HBDH) (195.50 U/L (IQR 190.25–212.00) to 181.00 U/L (IQR 165.75–187.25), P = 0.017) at 2 months. Notably, ST36 acupuncture and moxibustion also showed positive effects on sleep quality, knee joint function, and bowel movement patterns, and there were no syncope, severe pain or other adverse reactions. These findings provide clinical evidence that ST36 acupoint stimulation confers holistic health benefits for middle-aged and elderly populations, while maintaining an excellent safety profile.**

Keywords acupuncture and moxibustion; ST36; prophylactic treatment; low-density lipoprotein; uric acid

Introduction

Acupuncture and moxibustion is a fundamental component of traditional Chinese medicine (TCM) and has gained recognition as a prominent form of complementary and alternative medicine worldwide [1]. Zusanli, also known as Stomach 36 (ST36), is one of the well-known acupoints utilized in therapies of various diseases, as well as prophylactic treatment [2]. This highly versatile acupoint can regulate numerous physiologic functions [3–5]. It is usually employed in conjunction with other acupoints to address a range of health issues, and its clinical efficacy has been validated in clinical studies treating gastrointestinal diseases [6,7], degenerative osteoarthropathy [8], insomnia [9], and pain disorders [10,11].

The ST36 acupoint has also been documented in TCM for its potential effects on health maintenance and longevity when stimulated regularly. Recent studies have demonstrated that stimulation of the ST36 acupoint in mice results in the suppression of inflammation [12]. Electroacupuncture applied to the hindlimb ST36 acupoint, via PROKR2^{Cre}-marked sensory neurons, can activate the vagal-adrenal anti-inflammatory axis in mice [13]. Inflammation is recognized as a key driver of aging

Received August 19, 2025; accepted November 4, 2025
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[14,15]. The discovery of the anti-inflammatory effects of ST36 aligns with its alleged benefits for healthspan and lifespan [16–18]. However, these effects of ST36 have yet to be investigated in clinical studies.

In this study, we conducted a single-arm, open-label, pilot clinical trial. The objective was to evaluate the prophylactic effects of stimulating the ST36 acupoint through acupuncture and moxibustion in middle-aged and elderly participants. Our findings indicate that acupuncture and moxibustion at ST36 significantly reduces levels of low-density lipoprotein (LDL) and uric acid (UA) in individuals with abnormal LDL and UA values. Furthermore, stimulation of ST36 resulted in a significant decrease in the levels of lactic dehydrogenase (LDH) and α-hydroxybutyrate dehydrogenase (α-HBDH). Notably, acupuncture and moxibustion at ST36 exerts beneficial effects on sleep quality, knee joint function, and bowel movement patterns.

Methods

Study design

This single-arm, open-label, pilot clinical trial was conducted to examine the prophylactic effects of ST36 acupoint stimulation through acupuncture and moxibustion in middle-aged and elderly participants. The study was performed at the Haikou Hospital of Traditional Chinese Medicine in Haikou, Hainan Province, China, between October 12, 2023, and May 16, 2024. The total trial period was 6 months, and were divided into two stages. Stage 1 consisted of twice-weekly sessions over 2 months (16 sessions), while Stage 2 spanned 4 months, with a frequency of weekly sessions for the next 2 months (8 sessions), reducing to sessions once every 2 weeks for the final 2 months (4 sessions). Outcomes were assessed at baseline, 2 months, and 6 months. The assessment included the following biochemical parameters: LDL, UA, LDH, and α-HBDH, as well as measures of subjective sleep quality, knee joint function, and bowel movement patterns.

The trial was registered with Chinese Clinical Trial Registry (ChiCTR) under the identifier ChiCTR2500101531. The trial's protocol received ethical approval from the hospital's Ethics Committee (Approval number: HKSZYYYLL-2023 (Ke)-10). Participants provided written informed consent during the screening process, and the study adhered to the principles of the Declaration of Helsinki.

Participants

A total of 106 participants were recruited at Haikou Hospital of Traditional Chinese Medicine. The eligibility criteria were as follows: (1) aged 45–70 years, irrespective of gender; (2) with normal cognitive function, without communication disorders or severe underlying medical or psychiatric conditions; and (3) who voluntarily joined the study and provided written informed consent. The exclusion criteria were: (1) a history of severe coagulopathy or significant diseases such as cardiovascular, hepatic, or renal insufficiency, as well as serious physical or psychiatric conditions; (2) any pharmacological or non-pharmacological treatments within the past four weeks that could affect the study's outcomes; (3) localized skin infections, allergies, or other conditions at the acupoint area; (4) a history of contraindications to acupuncture, such as needle phobia; and (5) current involvement in other clinical trials.

Acupuncture and moxibustion treatments

Acupuncture and moxibustion was administered by licensed acupuncturists with at least 5 years of clinical experience. Acupoints were identified according to the Standard Acupuncture Nomenclature established by the World Health Organization (WHO) in 2014. Bilateral ST36 acupoints on the Stomach Meridian of Foot-Yangming were selected for the acupuncture and moxibustion intervention. These procedures adhered strictly to the WHO Benchmarks for the Practice of Acupuncture.

Acupuncture intervention

Acupuncture is a therapeutic technique that entails the insertion of fine, sterile metallic needles into specific acupoints to evoke *deqi* sensation, thereby triggering its therapeutic effects. Every participant was positioned in a supine posture. After identifying the acupoints, the skin at the acupoints and the acupuncturist's hands were disinfected with 75% alcohol. Disposable sterile acupuncture needles (0.25 mm × 50 mm, Huatuo, Suzhou Medical Supplies Co., Ltd., China) were used. Physicians pressed the needle holder tightly to the skin of the acupoints and then performed acupuncture. The needles were inserted perpendicularly into the acupoint to a depth of 20–30 mm at ST36, employing manual techniques such as lifting, thrusting, or rotating until the participant experienced the sensation of soreness, numbness, distention, or heaviness, which is referred to as *deqi*. The needles were retained for 30 min. Finally, the needle holes were pressed with a sterilized dry cotton swab briefly after needle removal.

Moxibustion intervention

Moxibustion is an external treatment method in TCM. This technique involves crushing dried leaves of *Artemisia argyi* H. Lév. and Vaniot (commonly known as

Aiye or moxa) and compressing them into cylindrical sticks. These prepared moxa sticks are then ignited and maintained at a specific distance above designated acupoints. The combustion stimulates the acupoints through thermal radiation, thereby producing both therapeutic and preventive effects. Moxa sticks (18 mm × 200 mm, Han Yi, Nanyang Hanyi Moxa Co., Ltd., China) were employed for mild suspended moxibustion at bilateral ST36. The moxa sticks were ignited and held 2–3 cm above the acupoints, ensuring that the participant felt warmth without discomfort or risk of burning. Moxibustion was applied for 30 min until a slight reddish mark appeared on the skin.

Intervention frequency and duration

Stage 1: twice a week for the initial 2 months, with each session lasting 30 min, totaling 16 sessions. Stage 2: during the third and fourth months, the frequency was reduced to once a week, totaling 8 sessions; during the fifth and sixth months, the frequency was further reduced to once every 2 weeks, maintaining a 30-min session duration, totaling 4 sessions.

Clinical assessments

General information was collected, including age, gender, height, weight, smoking status, drinking status, previous medical history, family medical history, and ethnicity.

Primary outcome

Biochemistry assay

Venous blood samples were collected from the antecubital fossa of everyone, who were reguired to come in the morning after an overnight fast. For biochemical analysis, 3 mL of blood was collected into serum separator tubes (SST) to allow clotting. Samples were processed immediately following collection. Biochemical analyses were conducted in the clinical laboratory of Haikou Hospital of Traditional Chinese Medicine.

The study outcome evaluated the variation in biochemical indicators assessed at three time points: at baseline, 2 months, and 6 months of treatment. These biochemical indicators included LDL, UA, LDH, and α-HBDH.

In our study, LDL, UA, LDH, and α-HBDH were measured on a BS-2800M auto biochemistry analyzer using kits obtained from Shenzhen Mindray Bio-Medical Electronics Co., Ltd. The testing of our indicators was standardized in accordance with the *Reference Intervals for Common Clinical Biochemistry Tests* Issued by the National Health Commission of the People's Republic of China. We listed the corresponding normal reference ranges in Table 1, and the details of the reagent kit manufacturers in Table 2. All assay results had coefficients of variation of < 5% between and within runs.

Secondary outcomes

Subjective sleep quality

The evaluation began with an assessment of subjective sleep quality, including indicators such as total sleep duration, sleep onset latency, and the frequency of awakenings.

Knee joint function

The knee joint function was evaluated across five domains: pain, stiffness, numbness, weakness, and functional limitations in daily activities. A visual analog scale (VAS), scaled from 0 to 10, was utilized to quantitate the level of knee joint functionality, with higher VAS scores denoted increased pain and compromised knee function.

Table 1 Normal reference ranges for primary indicators

Indicators	Male	Female
UA (μmol/L)	202–416	142–340
LDH (U/L)	120–250	120–250
α-HBDH (U/L)	72–182	72–182
LDL (mmol/L)	0–4.11	0–4.11

UA, uric acid; LDH, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; LDL, low-density lipoprotein.

Table 2 Reagents used in the study

Items	Batch No.	Specification	Manufacturer
LDL-C Assay Kit	142023006	R1: 4 × 58 mL, R2: 2 × 42 mL	Shenzhen Mindray Bio-Medical Electronics Co., Ltd.
α-HBDH Assay Kit	140523004	R1: 4 × 42 mL, R2: 4 × 12 mL	
LDH Assay Kit	142723005	R1: 4 × 42 mL, R2: 4 × 12 mL	
UA Assay Kit	141223006	R1: 6 × 57 mL, R2: 3 × 32 mL	

Instrument: modular fully automated biochemical analyzer (Model: BS-2800M), Shenzhen Mindray Bio-Medical Electronics Co., Ltd.

UA, uric acid; LDH, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; LDL, low-density lipoprotein.

Bowel movement patterns

The Bristol Stool Form Scale (BSFS) is a standardized 7-point ordinal scale widely employed in both clinical practice and research for assessing stool consistency [19]. It categorizes stool according to texture, ranging from the hardest (Type 1) to the softest (Type 7). It categorizes stool types as follows: 1 = separate hard lumps, like nuts (hard to pass); 2 = sausage-shaped but lumpy; 3 = like a sausage but with cracks on its surface; 4 = like a sausage or snake, smooth and soft; 5 = soft blobs with clear-cut edges (passed easily); 6 = fluffy pieces with ragged edges, a mushy stool; 7 = watery, no solid pieces entirely liquid. Stool types are further classified as hard (Types 1–2), normal (Types 3–5), and loose (Types 6–7).

Adverse events

We documented all treatment-induced adverse events, which were defined as any unfavorable or unintended signs, symptoms, or diseases related to the acupuncture and moxibustion treatment, such as acupuncture-induced hematoma, burn injury, syncope, infection, severe pain, and others. A severe adverse event was defined as any adverse event that poses a threat to a participant's life or functioning. The study investigators assessed adverse event severity (mild, moderate, or severe). Severe adverse events had to be reported to the principal investigator and safety monitoring board within 24 h after their occurrence. If adverse events were observed, the acupuncturists were required to assess the participant's condition to determine whether continuation of the treatment was feasible. This study described the number and proportion of adverse events observed.

Statistical analysis

The current single-arm pilot study employed an open-label exploration design. In the absence of preliminary data required for sample size estimation, this exploratory study proceeded without formal power calculation, per accepted pilot study methodologies [20]. Formal sample size calculation will be performed for subsequent definitive trials based on effect sizes observed in this study [21]. We referred to published articles on sample size calculation for pilot studies. One study [20] systematically analyzed 79 published pilot studies and reported a median sample size of 30 participants, suggesting this as the minimum requirement. Our pilot study enrolled 106 and 48 participants in the two phases respectively, both surpassing the recommended minimum sample size of 30 for feasibility studies.

Data were analyzed and plotted using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA). Continuous variables conforming to a normal distribution were expressed as mean ± standard deviation (SD), and non-normally distributed variables were expressed as median with interquartile range (IQR). Data were presented in various forms, including mean ± standard deviation, quartiles (Q1, Q3), or percentages (%). In this study, the collected data were divided into four categories of situations: normal before enrollment (2 months), abnormal before enrollment (2 months), normal before enrollment (6 months), and abnormal before enrollment (6 months). The changes in the indexes at two or three time points, "pre-acupuncture" (baseline), "2 months" (after 2 months of acupuncture and moxibustion), and "6 months" (after 6 months of treatment) were compared within each category, respectively. All data sets were tested for normality using the Shapiro–Wilk test. For normally distributed continuous data, paired *t*-tests were used for comparisons between two time points, and repeated-measures analysis of variance (ANOVA) was used for comparisons between three time points to determine whether the distributions of the indicators differed among the three time points. Further two-by-two comparisons were made if the overall test was significant (e.g., baseline vs. 2 months, baseline vs. 6 months, and 2 months vs. 6 months) to determine the source of the differences. Two-by-two comparisons of the 3 time points were performed using the Bonferroni test. The Wilcoxon signed-rank test was used for two-time point comparisons of non-normally distributed data, and the Friedman test was used for three-time point comparisons to determine whether there was a difference in the distributions of the indexes, and further two-by-two comparisons were made if the overall test was significant, and the Wilcoxon signed-rank test was used for two-by-two comparisons. In the comparison of two time points, $P \leqslant 0.05$ was considered statistically significant, and in the comparison of three time points, because three two-by-two comparisons were involved (baseline vs. 2 months, baseline vs. 6 months, and 2 months vs. 6 months), the corrected *P*-value of α was $0.05/3 \approx 0.0167$. $P \leqslant 0.017$ was considered statistically significant in the comparison of three time points.

Results

Baseline characteristics

A total of 106 participants were initially enrolled. During stage 1 (2-month ST36 acupoint acupuncture and moxibustion, twice weekly), 21 (19.8%) participants dropped out due to screening failures or discontinuation of the treatment, and 85 (80.2%) remained. Of these 85 participants, 48 participants proceeded to Stage 2 (4-month extended intervention), which involved weekly acupuncture and moxibustion at ST36 during months 3

and 4, followed by biweekly sessions through months 5 and 6, all 48 participants completed the full 6-month protocol with 0% dropout (Fig. 1). Table 3 presents the demographic and clinical characteristics of the 85 participants who completed the initial phase. The mean age of these participants was 57.15 ± 5.99 years, with 68 (80.0%) being women and 82 (96.5%) identifying as Han ethnicity. Additionally, 58 (68.2%) were retired, 6 (7.1%) were smokers, and 13 (15.3%) reported habitual alcohol consumption. Furthermore, 33 participants (38.8%) had a family medical history.

ST36 acupuncture and moxibustion effectively reduced LDL levels in individuals starting with abnormal LDL levels

Prior to the commencement of the clinical study, 34 out of 85 participants exhibited abnormal LDL levels. A statistically significant reduction in LDL levels was observed at the end of the first 2-month ST36 acupuncture and moxibustion intervention ($P < 0.001$). The median LDL value decreased from 4.51 mmol/L (IQR 4.27–5.09) to 4.18 mmol/L (IQR 3.77–4.76) (Table 4, Fig. 2A). In contrast, no significant reduction in LDL levels was noted in the remaining group of 51 individuals, who began with normal LDL values, after the same duration of the intervention ($P = 0.19$) (Table 4, Fig. 2B). The total results of the normality test are shown in Table S1.

Following the completion of the initial 2-month intervention, 48 individuals opted to continue treatment for an additional four months. Among them, 18 individuals who had abnormal LDL levels prior to the clinical study experienced a statistically significant reduction in their LDL levels. Specifically, their median LDL levels decreased from 4.46 mmol/L (IQR 4.32–4.71) to 3.97 mmol/L (IQR 3.32–4.47) by the end of the intervention ($P = 0.004$) (Table 4, Fig. 2C). The LDL level of 3.97 mmol/L falls within the normal range (≤ 4.11 mmol/L) established by the testing system. Conversely, the remaining 30 participants, who had baseline LDL levels within the normal range, showed no significant changes by the end of the intervention (Table 4, Fig. 2D).

ST36 acupuncture and moxibustion effectively decreased UA levels in individuals with abnormal UA levels

Of the 85 individuals in the first intervention period, 30 individuals with abnormal UA levels experienced a significant reduction, with the median levels decreasing from 401.00 μmol/L (IQR 363.50–485.75) to 378.00 μmol/L (IQR 338.75–430.00) ($P < 0.001$) (Table 4, Fig. 3A). However, no statistically significant change was observed in the UA levels of the remaining 55 individuals whose UA levels fell within the normal range (male 202–416 μmol/L; female 142–340 μmol/L) ($P = 0.574$) (Table 4, Fig. 3B).

Of the 48 individuals in the Stage 2 intervention period, 15 individuals with abnormal UA levels experienced a significant reduction, with the median levels decreasing

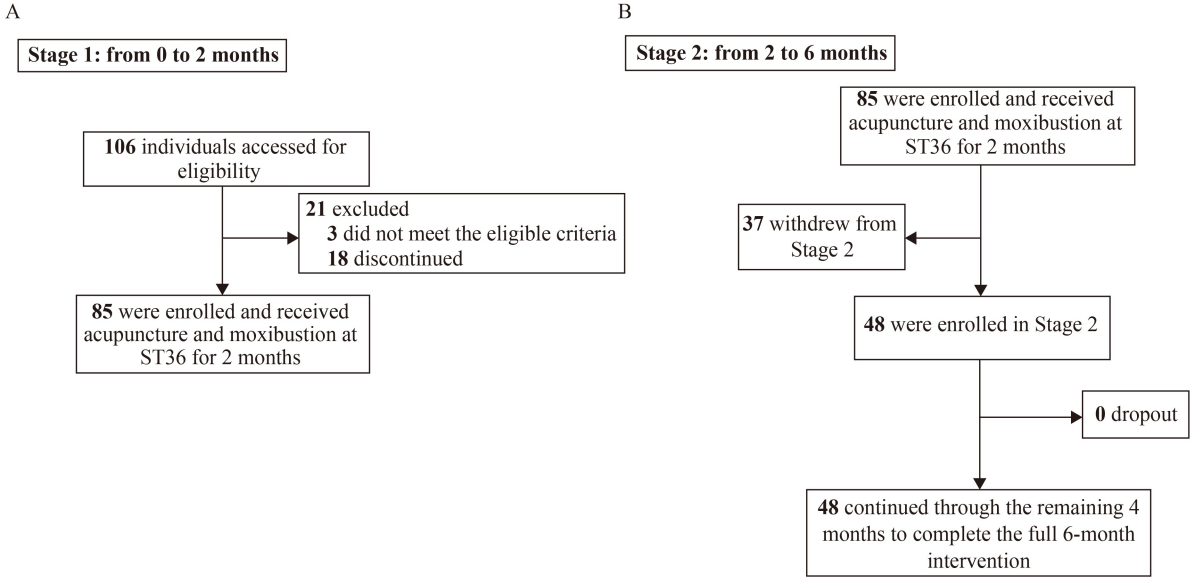

Fig. 1 Consort flow diagram. The study diagram illustrates the participants' disposition process, encompassing screening, and consolidation treatment. (A) Stage 1: initial 2-month intervention involving acupuncture and moxibustion at the ST36 acupoint, administered twice weekly. (B) Stage 2: following the completion of Stage 1, participants received follow-up treatment consisting of one-weekly acupuncture and moxibustion at ST36 for 2 months, followed by sessions once every 2 weeks for an additional 2 months.

Table 3 Characteristics of the participants

Characteristics	Baseline (n = 85)	Post-2 months period (n = 48 (48/85)[a])
Mean age, year (95% CI)	57.15 (55.86–58.45)	57.56 (56.05–59.08)
Gender, *n* (%)		
Female	68 (80.0)	37 (77.1)
Male	17 (20.0)	11 (22.9)
Mean BMI, kg/m^2 (95% CI)	24.21 (23.57–24.86)	24.41 (23.52–25.29)
Employment status, *n* (%)		
Retired	58 (68.2)	38 (79.2)
Unretired	27 (31.8)	10 (20.8)
Smoking status, *n* (%)	6 (7.1)	5 (10.4)
Drinking status, *n* (%)	13 (15.3)	5 (10.4)
Previous medical history, *n* (%)		
Yes	30 (35.3)	20 (41.7)
No	55 (64.7)	28 (58.3)
Family medical history, *n* (%)		
Yes	33 (38.8)	18 (37.5)
No	52 (61.2)	30 (62.5)
Ethnic group, *n* (%)		
Han	82 (96.5)	47 (97.9)
Hui	1 (1.2)	1 (2.1)
Li	1 (1.2)	0
She	1 (1.2)	0

CI, confidence interval; BMI, body mass index.
[a]Among the initial 85 participants, 48 agreed to continue the following 4-month treatment.

from 399.00 μmol/L (IQR 356.00–483.00) to 361.00 μmol/L (IQR 323.00–421.00). This change was statistically significant (P = 0.003) (Table 4, Fig. 3C). In contrast, among the remaining 33 individuals with normal UA levels, no significant changes were observed (Table 4, Fig. 3D).

ST36 acupuncture and moxibustion effectively reduced LDH and α-HBDH levels

As illustrated in Fig. 4A and 4B, prior to the treatment, 74 out of 85 individuals had normal LDH levels (120–250 U/L) before the intervention, with a median level of 203.00 U/L (IQR 185.00–220.00), which significantly decreased to 189.00 U/L (IQR 172.25–203.00) (P < 0.001) after 2-month acupuncture and moxibustion intervention. Similarly, among the 85 participants, 11 individuals with abnormal LDH levels, initially at a median level of 262.00 U/L (IQR 253.00–279.00), showed a significant reduction to a median level of 233.00 U/L (IQR 205.00–240.00) (P < 0.001). In Stage 2, among 48 individuals, 6 exhibited abnormal LDH levels, which showed a marked reduction. The median LDH level decreased from 266.50 U/L (IQR 257.50–282.25) to 235.50 U/L (IQR 215.50–244.75), with a statistically significant difference (P < 0.001) (Table 4, Fig. 4C). Furthermore, among these 48 participants, 42 individuals with normal LDH levels also experienced a significant decline, with the median levels decreasing from 207.50 U/L (IQR 195.75–220.50) to 186.50 U/L (IQR 176.75–202.25) (P < 0.001) (Table 4, Fig. 4D).

Besides, in the Stage 1 intervention, regardless of whether the volunteers were normal or abnormal before enrollment, the overall average level of α-HBDH significantly decreased (P < 0.001) (Table 4, Fig. 5A and 5B). The normal (n = 68) α-HBDH levels before enrollment decreased from 153.00 U/L (IQR 135.00–163.75) to 141.50 U/L (IQR 127.00–150.75), and the abnormal (n = 17) α-HBDH levels before enrollment decreased from 193.00 U/L (IQR 190.50–212.00) to 180.00 U/L (IQR 157.50–188.50), which is already at the upper limit of the normal range. Based on the above results, the Stage 2 intervention was continued. At this time, there were 40 individuals with normal α-HBDH level before enrollment. The α-HBDH level was 156.50 U/L (IQR 137.75–167.75) at baseline, at 2 months of acupuncture and moxibustion was 143.00 U/L (IQR 132.50–154.25), and at 6 months of acupuncture and moxibustion was 145.50 U/L (IQR 137.25–157.75). Compared with the baseline, the decline at 2 months of

Table 4 LDL, UA, LDH, and α-HBDH levels of acupuncture and moxibustion at ST36 from baseline to 2 months and 6 months

Indicators	*n*	*N* = 85			*n*	*N* = 48					
		Baseline	2 months	*P* value		Baseline	2 months	6 months	*P* value		
		M (P_{25}, P_{75})	*M* (P_{25}, P_{75})	Baseline vs. 2 months		*M* (P_{25}, P_{75})	*M* (P_{25}, P_{75})	*M* (P_{25}, P_{75})	Baseline vs. 2 months	Baseline vs. 6 months	2 months vs. 6 months
LDL, mmol/L											
Abnormal	34	4.51 (4.27, 5.09)	4.18 (3.77, 4.76)	< 0.001	18	4.46 (4.32, 4.71)	4.16 (3.70, 4.87)	3.97 (3.32, 4.47)	0.102	0.004	0.306
Normal	51	3.34 (2.96, 3.71)	3.25 (2.73, 3.57)	0.190	30	3.22 (2.93, 3.70)	3.05 (2.70, 3.57)	3.25 (2.92, 3.58)	0.106	0.050	0.056
UA, μmol/L											
Abnormal	30	401.00 (363.50, 485.75)	378.00 (338.75, 430.00)	< 0.001	15	399.00 (356.00, 483.00)	387.00 (350.00, 436.00)	361.00 (323.00, 421.00)	0.038	0.003	0.069
Normal	55	299.00 (256.00, 318.00)	294.00 (253.00, 328.00)	0.574	33	301.00 (265.50, 317.50)	295.00 (266.00, 330.50)	310.00 (261.50, 334.50)	0.872	0.489	0.133
LDH, U/L											
Abnormal	11	262.00 (253.00, 279.00)	233.00 (205.00, 240.00)	< 0.001	6	266.50 (257.50, 282.25)	237.00 (232.75, 240.50)	235.50 (215.50, 244.75)	0.0015	< 0.001	1.000
Normal	74	203.00 (185.00, 220.00)	189.00 (172.25, 203.00)	< 0.001	42	207.50 (195.75, 220.50)	194.00 (179.00, 203.00)	186.50 (176.75, 202.25)	< 0.001	< 0.001	0.027
α-HBDH, U/L											
Abnormal	17	193.00 (190.50, 212.00)	180.00 (157.50, 188.50)	< 0.001	8	195.50 (190.25, 212.00)	181.00 (165.75, 187.25)	183.00 (170.00, 195.75)	0.017	0.070	1.000
Normal	68	153.00 (135.00, 163.75)	141.50 (127.00, 150.75)	< 0.001	40	156.50 (137.75, 167.75)	143.00 (132.50, 154.25)	145.50 (137.25, 157.75)	0.001	0.027	0.468

M, median; P_{25}, 25th percentile; P_{75}, 75th percentile; LDL, low-density lipoprotein; UA, uric acid; LDH, lactic dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; normal, normal physical examination indicators before enrollment; abnormal, pre-enrollment physical examination indicators are not within normal range.

For comparisons between pre-acupuncture and 2 months post-acupuncture, data on LDL, UA, LDH, and α-HBDH that followed a normal distribution were analyzed using the paired-sample *t*-test, while data that did not follow a normal distribution were analyzed using the Wilcoxon signed-rank test, with $P \leqslant 0.05$ considered statistically significant.

For comparisons among pre-acupuncture, 2 months post-acupuncture, and 6 months post-acupuncture, normally distributed data were analyzed using repeated-measures ANOVA for overall testing with pairwise comparisons performed by the Bonferroni test, whereas non-normally distributed data were analyzed using the Friedman test for overall testing with pairwise comparisons performed by the Wilcoxon signed-rank test, and in this case, $P \leqslant 0.017$ was considered statistically significant.

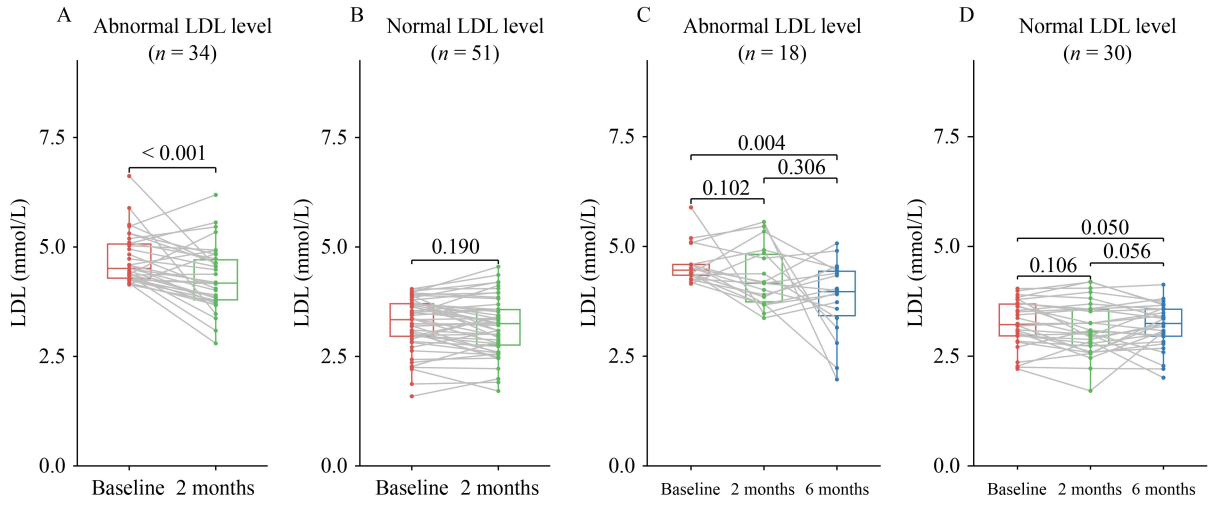

Fig. 2 Changes in abnormal and normal LDL levels. (A) LDL levels before and after the Stage 1 acupuncture and moxibustion at ST36 in individuals starting with abnormal LDL test result. Data were normally distributed (Shapiro–Wilk test); paired-sample *t*-test was used. $P \leqslant 0.05$ was considered significant. (B) LDL levels before and after the Stage 1 acupuncture and moxibustion at ST36 in individuals starting with normal LDL test result. Data were normally distributed (Shapiro–Wilk test); paired samples *t*-test was used. $P \leqslant 0.05$ was considered significant. (C) LDL levels before and after the Stage 1 and 2 acupuncture and moxibustion at ST36 in individuals starting with abnormal LDL test result. Data were not normally distributed (Shapiro–Wilk test); overall analysis by Friedman test ($P \leqslant 0.05$), pairwise comparisons by Wilcoxon signed-rank test ($P \leqslant 0.017$). (D) LDL levels at baseline, after Stage 1, and after Stage 2 acupuncture and moxibustion at ST36 in individuals starting with normal LDL test result. Data were normally distributed (Shapiro–Wilk test); overall analysis by repeated-measures ANOVA ($P \leqslant 0.05$), pairwise comparisons by Bonferroni test ($P \leqslant 0.017$).

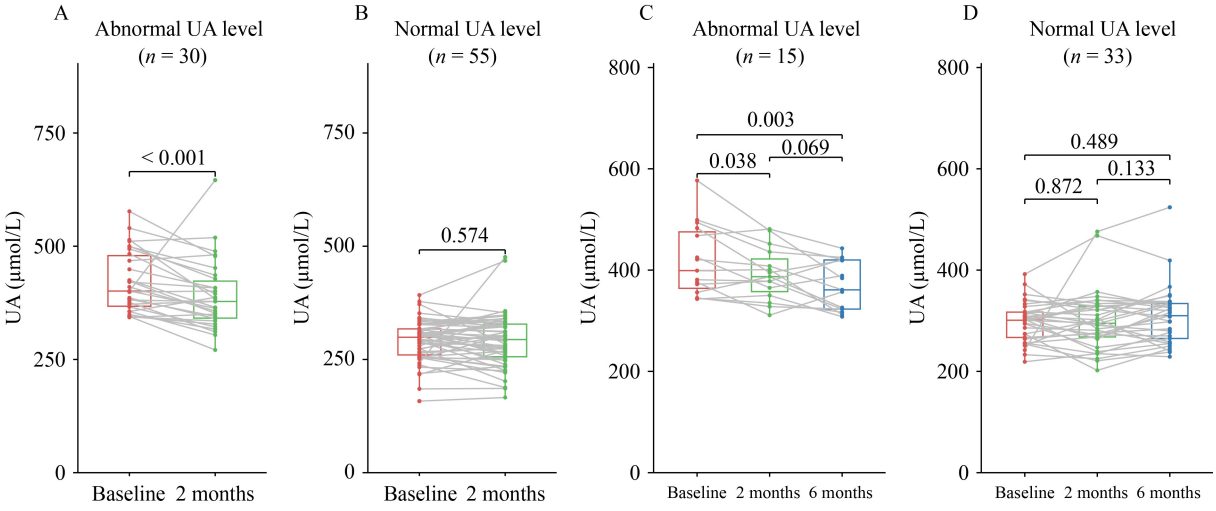

Fig. 3 Changes in abnormal and normal UA levels. (A) UA levels at baseline and after Stage 1 acupuncture and moxibustion at ST36 in individuals starting with abnormal UA test results. Data were not normally distributed (Shapiro–Wilk test); analyzed using Wilcoxon signed-rank test. $P \leqslant 0.05$ was considered significant. (B) UA levels at baseline and after Stage 1 acupuncture and moxibustion at ST36 in individuals starting with normal UA test result. Data were not normally distributed (Shapiro–Wilk test); analyzed using Wilcoxon signed-rank test. $P \leqslant 0.05$ was considered significant. (C) UA levels at baseline, after Stage 1, and after Stage 2 acupuncture and moxibustion at ST36 in individuals starting with abnormal UA test result. Data were not normally distributed (Shapiro–Wilk test); overall analysis by Friedman test ($P \leqslant 0.05$), pairwise comparisons by Wilcoxon signed-rank test ($P \leqslant 0.017$). (D) UA levels at baseline, after Stage 1, and after Stage 2 acupuncture and moxibustion at ST36 in individuals starting with normal UA test result. Data were not normally distributed (Shapiro–Wilk test); overall analysis by Friedman test ($P \leqslant 0.05$), pairwise comparisons by Wilcoxon signed-rank test ($P \leqslant 0.017$).

acupuncture and moxibustion had a statistical difference ($P = 0.001$) (Table 4, Fig. 5D), while the decline at 6 months of acupuncture and moxibustion had no statistical difference ($P = 0.027 > 0.017$), and the level of α-HBDH at 6 months of acupuncture and moxibustion was slightly higher than that at 2 months of acupuncture and moxibustion. There were 8 people with abnormal α-HBDH level before enrollment. The α-HBDH level was

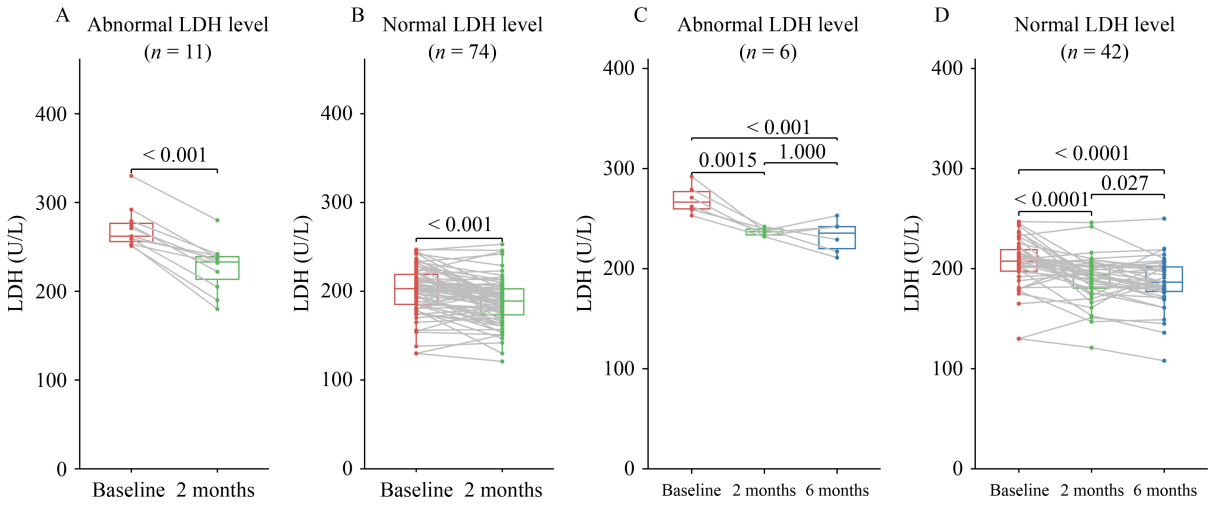

Fig. 4 Changes in abnormal and normal LDH levels. (A) LDH levels at baseline and after the Stage 1 acupuncture and moxibustion at ST36 in individuals starting with abnormal LDH test result. Data were normally distributed (Shapiro–Wilk test); analyzed using paired-sample *t*-test. $P \leqslant 0.05$ was considered significant. (B) LDH levels baseline and after the Stage 1 acupuncture and moxibustion at ST36 in individuals starting with normal LDH test result. Data were normally distributed (Shapiro–Wilk test); analyzed using paired-sample *t*-test. $P \leqslant 0.05$ was considered significant. (C) LDH levels at baseline, after the Stage 1, and after the Stage 2 acupuncture and moxibustion at ST36 in individuals starting with abnormal LDH test result. Data were normally distributed (Shapiro–Wilk test); overall analysis by repeated-measures ANOVA ($P \leqslant 0.05$), pairwise comparisons by Bonferroni test ($P \leqslant 0.017$). (D) LDH levels at baseline, after the Stage 1, and after the Stage 2 acupuncture and moxibustion at ST36 in individuals starting with normal LDH test result. Data were not normally distributed (Shapiro–Wilk test); overall analysis by Friedman test ($P \leqslant 0.05$), pairwise comparisons by Wilcoxon signed-rank test ($P \leqslant 0.017$).

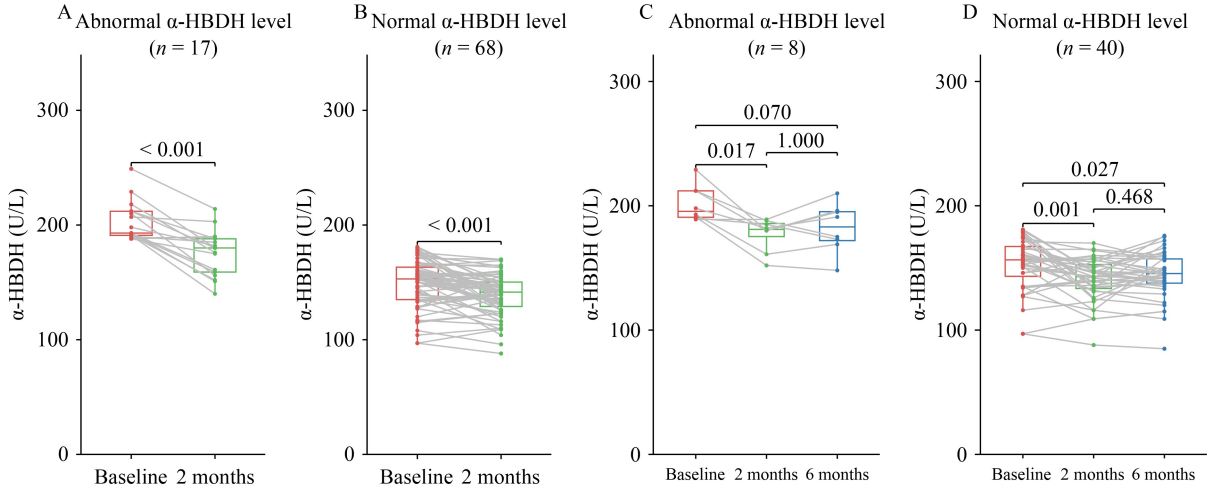

Fig. 5 Changes in abnormal and normal α-HBDH levels. (A) α-HBDH levels at baseline and after the Stage 1 acupuncture and moxibustion at ST36 in individuals starting with abnormal α-HBDH test result. Data were normally distributed (Shapiro–Wilk test); analyzed using paired-sample *t*-test. $P \leqslant 0.05$ was considered significant. (B) α-HBDH levels at baseline and after the Stage 1 acupuncture and moxibustion at ST36 in individuals starting with normal α-HBDH test result. Data were normally distributed (Shapiro–Wilk test); analyzed using paired-sample *t*-test. $P \leqslant 0.05$ was considered significant. (C) α-HBDH levels at baseline, after the Stage 1, and after the Stage 2 acupuncture and moxibustion at ST36 in individuals starting with abnormal α-HBDH test result. Data were normally distributed (Shapiro–Wilk test); overall analysis by repeated-measures ANOVA ($P \leqslant 0.05$), pairwise comparisons by Bonferroni test ($P \leqslant 0.017$). (D) α-HBDH levels at baseline, after the Stage 1, and after the Stage 2 acupuncture and moxibustion at ST36 in individuals starting with normal α-HBDH test result. Data were not normally distributed (Shapiro–Wilk test); overall analysis by Friedman test ($P \leqslant 0.05$), pairwise comparisons by Wilcoxon signed-rank test ($P \leqslant 0.017$).

195.50 U/L (IQR 190.25–212.00) at baseline, 181.00 U/L (IQR 165.75–187.25) at 2 months of acupuncture and moxibustion, and 183.00 U/L (IQR 170.00–195.75) at 6 months of acupuncture and moxibustion. The change trend was consistent with the normal before enrollment (Table 4, Fig. 5C).

Subjective symptom outcomes

Improvement in sleep quality

Among the participants, 37 participants reported improvements in sleep quality at both the 2-month and 6-month follow-ups. Compared with baseline, significant and consistent enhancements were observed across multiple sleep parameters. Specifically, total sleep duration increased from (5.72 ± 1.08) h to (6.69 ± 1.03) h at 2 months ($P < 0.001$, $n = 37$) and from their baseline of (5.56 ± 1.11) h to (6.61 ± 1.10) h at 6 months ($P < 0.001$, $n = 27$). In parallel, sleep onset latency decreased substantially, from (47.43 ± 27.68) min to (27.84 ± 20.02) min at 2 months ($P < 0.001$, $n = 37$) and from (46.30 ± 24.11) min to (24.11 ± 14.56) min at 6 months ($P < 0.001$, $n = 27$). Furthermore, nighttime awakenings were significantly reduced, with the proportion of participants reporting no awakenings rising from 22 (59.5%) to 26 (70.3%) at 2 months ($P = 0.007$, $n = 37$) and from 14 (51.9%) to 22 (81.5%) at 6 months ($P = 0.001$, $n = 27$) (Table 5).

Knee function improvement

With respect to knee joint function, 16 participants reported perceived improvements during the study period. Notably, VAS pain scores declined significantly (2 months: 3.19 ± 3.31 to 1.63 ± 1.67, $P = 0.007$, $n = 16$; 6 months: 3.00 ± 2.97 to 0.82 ± 1.08, $P = 0.011$, $n = 11$). Likewise, both stiffness and weakness demonstrated significant reductions (stiffness: 2 months $P = 0.041$, $n = 16$;

Table 5 Variations in sleep quality, knee joint function, and bowel movement patterns with acupuncture and moxibustion at ST36 from baseline to 2 and 6 months

Outcome assessments	2-month period ($N = 85$)		P value (baseline vs. 2 months)	6-month period ($N = 48$)		P value (baseline vs. 6 months)
	Baseline	2 months		Baseline	6 months	
Sleep quality						
Participants with perceived changes in sleep quality, *n*	37	37		27	27	
Total sleep duration, h (mean ± SD)	5.72 ± 1.08	6.69 ± 1.03	< 0.001	5.56 ± 1.11	6.61 ± 1.10	< 0.001
Sleep onset latency, min (mean ± SD)	47.43 ± 27.68	27.84 ± 20.02	< 0.001	46.30 ± 24.11	24.11 ± 14.56	< 0.001
Number of sleep awakenings, *n* (%)			0.007			0.001
No awakening	22 (59.46)	26 (70.27)		14 (51.85)	22 (81.48)	
1–2 awakenings	4 (10.81)	10 (27.03)		2 (7.41)	5 (18.52)	
3 awakenings	8 (21.62)	1 (2.70)		8 (29.63)	0 (0.00)	
More than 3 awakenings	3 (8.11)	0 (0.00)		3 (11.11)	0 (0.00)	
Knee joint function						
Participants with perceived changes in knee function, *n*	16	16		11	11	
Pain, (mean ± SD)	3.19 ± 3.31	1.63 ± 1.67	0.007	3.00 ± 2.97	0.82 ± 1.08	0.011
Stiffness, (mean ± SD)	2.75 ± 3.40	1.25 ± 1.29	0.041	2.45 ± 3.14	0.91 ± 1.04	0.043
Numbness, (mean ± SD)	1.69 ± 2.80	0.75 ± 0.93	0.104	1.45 ± 2.95	0.45 ± 0.52	0.197
Weakness, (mean ± SD)	3.63 ± 2.85	1.38 ± 1.41	0.005	3.36 ± 3.08	0.64 ± 0.51	0.018
Difficulty with activities, (mean ± SD)	2.19 ± 2.11	1.25 ± 1.16	0.017	2.18 ± 2.27	0.73 ± 0.91	0.026
Bowel movement patterns						
Participants with perceived changes in bowel movement patterns, *n*	21	21		13	13	
Frequency of defecation, *n* (%)			0.681			0.245
More than once daily	10 (47.62)	9 (42.86)		6 (46.15)	8 (61.54)	
Once daily	7 (33.33)	10 (47.62)		3 (23.08)	5 (38.46)	
Every 2–3 days	3 (14.29)	2 (9.52)		3 (23.08)	0 (0.00)	
Less than once every 3 days	1 (4.76)	0 (0.00)		1 (7.69)	0 (0.00)	
Stool consistency, *n* (%)			0.001			0.019
Hard	5 (23.81)	3 (14.29)		3 (23.08)	1 (7.69)	
Normal	5 (23.81)	16 (76.19)		4 (30.77)	11 (84.62)	
Loose stools	11 (52.38)	2 (9.52)		6 (46.15)	1 (7.69)	

6 months $P = 0.043$, $n = 11$; weakness: 2 months $P = 0.005$, $n = 16$; 6 months $P = 0.018$, $n = 11$). Moreover, difficulties with daily activities were also significantly alleviated (2 months: 2.19 ± 2.11 to 1.25 ± 1.16, $P = 0.017$, $n = 16$; 6 months: 2.18 ± 2.27 to 0.73 ± 0.91, $P = 0.026$, $n = 11$). Although numbness tended to decrease over time, these changes did not reach statistical significance (Table 5).

Enhancements in bowel movement patterns

Regarding bowel movement patterns, 21 participants experienced perceived changes over the study course. While no significant differences in defecation frequency were observed between baseline and follow-up ($P > 0.05$), stool consistency demonstrated a clear normalization trend. Specifically, the proportion of participants reporting normal stool consistency increased from 5 (23.8%) to 16 (76.2%) at 2 months ($P = 0.001$, $n = 21$) and from 4 (30.8%) to 11 (84.6%) at 6 months ($P = 0.019$, $n = 13$). Correspondingly, reports of hard and loose stools decreased substantially over time (Table 5).

Adverse events

Throughout the entire duration of the acupuncture and moxibustion intervention, no severe adverse events were reported. Hematomas, the most common self-reported acupuncture-related AEs, occurred in 1.18% of participants during the acupuncture and moxibustion period (Table 6). All AEs were effectively managed by healthcare professionals in a hospital setting, and no participants discontinued the acupuncture and moxibustion intervention due to these events.

Table 6 Adverse events

Variables	*n* (%)[a]
Acupuncture-induced hematoma	1 (1.18)
Minor burn injury	0 (0.00)
Syncope	0 (0.00)
Infection	0 (0.00)
Severe pain	0 (0.00)
Others	0 (0.00)

[a]Values are numbers (percentages).

Discussion

This clinical trial was designed as an open-label, exploratory pilot study, aiming to evaluate the prophylactic efficacy of acupuncture and moxibustion at ST36 in middle-aged and elderly individuals. The study demonstrated that participants with abnormal LDL levels experienced a significant reduction in LDL levels after 2 and 6 months of acupuncture and moxibustion at the ST36 acupoint, with a more pronounced reduction observed at 6 months; and at 6 months, their LDL levels decreased to within the normal range. Remarkably, even with the decreased frequency of acupuncture and moxibustion sessions after 2 months, acupuncture and moxibustion at ST36 continued to effectively lower LDL levels. In contrast, participants with normal LDL levels exhibited no significant changes in LDL at either time point.

Existing studies have found that elevated levels of LDL cholesterol can lead to hyperlipidemia, which is a major risk factor for cardiovascular diseases (CVDs). Mechanistically, excessive LDL particles accumulate in the arterial wall to promote the formation of atherosclerotic plaque, further increasing the risk of CVDs. Therefore, the increase of LDL cholesterol level is associated with the increase of incidence rate of CVDs [22,23]. Epidemiological surveys show that approximately 523 million people worldwide are currently affected by CVDs, with China alone accounting for 330 million cases [24], and the prevalence of dyslipidemia in middle-aged and elderly Chinese populations is 43.0%, with specific rates of 34.6% for abnormal LDL [25]. Importantly, a meta-analysis of 26 trials reveals that a reduction of 1.0 mmol/L in LDL cholesterol over one year correlates with a 22% decrease in major vascular events [26]. These findings underscore the crucial public health benefit of researching and implementing effective strategies to manage LDL cholesterol levels. Currently, statins are the cornerstone of lipid-lowering therapy and are highly effective in reducing plasma LDL levels. However, like most medications, statins may have adverse effects. The most common adverse effects include myopathy, rhabdomyolysis, and elevated liver enzymes levels, with the risk increasing at higher doses [27]. Given these limitations, exploring alternative or complementary interventions is warranted. Based on our results, acupuncture and moxibustion at the single acupoint of ST36 can effectively reduce LDL levels. Consequently, this intervention may serve as a promising complementary approach to LDL reduction, potentially contributing to a decreased incidence of major vascular events.

In addition, we have found that after completing the 6-month treatment, individuals with abnormal UA levels experienced a significant reduction, while no significant changes were observed in individuals with normal UA levels. UA is the final product of purine nucleotide catabolism, and its physiologic role is highly complex and multifaceted [28]. At normal concentrations, UA exhibits remarkable antioxidant properties, effectively mitigating oxidative stress caused by free radicals and reactive

oxygen species (ROS). Furthermore, UA also reduces the release of inflammatory factors (e.g., TNF-α, IL-6) by decreasing NF-κB activation. These two effects have potential neuroprotective and anti-aging effects [28–30]. However, an imbalance in UA levels can alter its original antioxidant or anti-inflammatory effects [31]. For example, when UA concentrations exceed physiologic levels, it will turn into an oxidant —promoting ROS production, activating NLRP3 inflammasome, and intensifying inflammation, thus increasing the risk of gout, type II diabetes, CVDs, and other diseases [28]. To address the harm caused by excessive UA to the human body, the current management strategies include two primary approaches: non-pharmacological and pharmacological interventions [32]. Non-pharmacological strategies, which are especially important for managing hyperuricemia in asymptomatic individuals, include dietary modifications, cessation of smoking and alcohol, weight loss, and regular exercise [33]. Pharmacological interventions primarily involve the use of urate-lowering drugs and emerging therapeutic compounds [34,35]. However, elevated UA levels remain inadequately managed, largely due to factors such as suboptimal dosing of urate-lowering therapies, challenges in adherence to behavioral interventions, and intolerance or adverse events [28,36]. Thus, developing alternative treatment options that can effectively and safely reduce serum UA levels remains an urgent clinical priority. Acupuncture and moxibustion at ST36 have been shown in our clinical trials to effectively reduce UA levels without adversely affecting individuals with normal UA levels. Based on these results, acupuncture and moxibustion at ST36 may serve as a viable non-pharmacological adjunctive therapy to conventional UA-lowering treatments.

LDH is a key enzyme in energy metabolism. Serum α-HBDH is an isoenzyme of LDH [37], both of which are serum markers for myocardial injury [38], hemolysis, liver disease [39], and certain malignant tumors [40,41]. Multiple studies have found that high LDH levels not only promotes cancer cell aggressiveness but also predicts poor clinical outcomes. Compared with patients with low LDH levels, tumor patients with elevated LDH levels have significantly shorter disease-free survival and overall survival, a 10-fold increased risk of disease recurrence, and a 4-fold increased risk of death [42,43]. Measurement of LDH levels has become a standard tool for monitoring cancer treatment efficacy [44]. Additionally, according to published data, several small-molecule LDH inhibitors can effectively limit tumor progression and enhance antitumor immunity, and have been used as anticancer drugs in clinical trials [41,45–47].

In this study, during the 6-month acupuncture and moxibustion intervention, subjects with either normal or abnormal baseline serum LDH levels exhibited a statistically significant continuous decrease in LDH concentrations, with their serum LDH levels stabilizing within the normal range. Meanwhile, compared with the baseline, the subjects with normal and abnormal initial α-HBDH levels showed a general downward trend after 2 months of acupuncture and moxibustion, almost falling to the normal range, but no further decline after 6 months of acupuncture and moxibustion. This reveals a time-dependent efficacy of ST36 stimulation: effective for acute α-HBDH reduction but with limited sustainability during chronic intervention. Such temporal dynamics suggest that intermittent intensive treatment cycles may optimize long-term α-HBDH control.

Our findings suggest investigating acupuncture and moxibustion as potential interventions for elevated LDH and α-HBDH levels. Given these markers' established roles as biomarkers for myocardial injury [38], hepatic dysfunction [39], and tumor progression [40]. This LDH-lowering effect positions acupuncture and moxibustion as a potential strategy to intercept pre-pathological states characterized by LDH-driven metabolic dysregulation.

This study also collected data on participants' subjective symptoms, indicating that the acupuncture and moxibustion at ST36 was associated with substantial improvements in sleep quality, significant enhancements in knee joint function, and noteworthy positive effects on bowel movement patterns —including improved stool consistency and relief from constipation. These findings align with prior research. For example, studies have found that ST36, recognized as the "He Acupoint" of the stomach meridian, is the most frequently used acupoint in the clinical treatment of functional dyspepsia [48], postprandial distress syndrome [7], postoperative ileus [49], diarrhea [50], and other gastrointestinal diseases [51]. Furthermore, clinical trials investigating acupuncture and moxibustion for sleep disturbances have yielded results consistent with our findings [9,52], demonstrating significant improvements in sleep efficiency, prolonged total sleep duration, and reduced frequency of nighttime awakenings. Remarkably, our study expands previous research, which shows that acupuncture and moxibustion has efficacy in improving Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores related to chronic knee pain and the functional limitations associated with osteoarthritis [8]. Similarly, our study also corroborates acupuncture and moxibustion's effectiveness in alleviating knee pain and enhancing knee function, even when evaluated through subjective measures. Given that low-grade inflammation is pivotal in osteoarthritis pathogenesis [53], we propose that the observed improvements following ST36 acupuncture and moxibustion are mediated by its anti-inflammatory properties. These findings highlight the potential of acupuncture and moxibustion at ST36 in improving sleep

quality, knee joint function, and bowel movement patterns, and provide clinical data for future research on ST36.

Current clinical studies often employ semi-standardized or multi-acupoint treatment protocols, with a prevailing consensus among researchers and practitioners of TCM that the application of a single acupoint may result in relatively limited efficacy. However, our study revealed that, despite the exclusive use of the ST36 acupoint, therapeutic effects were still evident. This observation suggests that a single acupoint can indeed yield effective outcomes, potentially providing a clearer understanding of the underlying mechanisms associated with acupuncture and moxibustion. This phase 1/2 pilot study lays a foundation for the design of a future prospective, multicenter, randomized controlled clinical trial. In line with these promising findings, we observed that most participants exhibited a significant decrease in abnormal indicators following the intervention, and this trend was statistically significant. However, a small proportion of participants showed an increase in their indicators of post-intervention. A plausible explanation for this is, according to TCM, acupuncture and moxibustion have certain contraindications, and not all individuals are suitable for long-term stimulation of the ST36 acupoint. Therefore, individualized assessments should be conducted before using ST36 as a prophylactic treatment point. Alternatively, another potential explanation is that, although participants were instructed to maintain healthy lifestyle habits during the intervention, continuous monitoring of their behaviors was not feasible. Some participants may have altered their daily habits or experienced common health issues, such as alcohol consumption, irregular sleep patterns, or colds, which could introduce variability and potentially affect the results.

Despite the promising findings, this study has several limitations. First, it was not conducted as a randomized controlled trial, which may have introduced selection bias and potentially compromised the accuracy of the outcome assessments. Second, as an open-label, exploratory pilot trial, the study did not define pre-specified primary outcomes at its inception. This methodological approach precludes rigorous sample size calculation and consequently increases the risk of Type II error due to insufficient statistical power. This study involves multiplicity issues, and no correction was performed across endpoints or subgroups. However, such adjustments will be addressed in future RCTs. Third, some efficacy indicators relied on subjective assessments, which may have introduced bias into the results, and the study also did not explore the molecular mechanisms underlying the effects of ST36 single-point stimulation and lacked long-term follow-up data. Nevertheless, the encouraging efficacy observed in this study suggests the necessity for further investigation and validation through more rigorous, controlled trials.

One of the key challenges in designing randomized controlled trials (RCTs) to evaluate the prophylactic health benefits of TCM lies in the absence of clearly defined, objective, and universally accepted clinical endpoints that can reliably reflect preventive effects in generally healthy individuals. This issue is particularly pronounced in the context of TCM [54]. As a result, it is especially difficult to construct RCTs with fixed, objective endpoints that align with TCM principles while meeting the methodological rigor expected in evidence-based research. Consequently, our study was designed as an open-label, exploratory pilot, aiming to observe potential health-promoting effects of acupuncture and moxibustion at ST36 in middle-aged and elderly individuals, with the primary objective of identifying physiologic or clinical change patterns that may inform endpoint selection for future RCTs. For example, if reductions in LDL or UA levels are consistently observed, these may become the focus of targeted, hypothesis-driven RCTs in the future.

Conclusions

The results of this phase 1/2 pilot trial suggest that acupuncture and moxibustion applied at the ST36 acupoint may serve as an effective prophylactic intervention for health maintenance in middle-aged and elderly populations, while also demonstrating a favorable safety profile.

Acknowledgements

We sincerely thank the kind editor and the reviewers for improving of our manuscript. We thank the patients, health care professionals, clinical staff, research staff, study therapists, and patient/stakeholder partners at all study sites for their contributions to this study. This work was supported by the Key Research and Development Project of Hainan Province (No. 2023ICAC-YANFA), the Science and Technology Platform Construction Project of Hainan Province (Nos. 2023ICAC-YUNXING and 2024ICAC-YUNXING), Regional Project of National Natural Science Foundation of China (No. 82060898), Hainan Provincial Natural Science Foundation High-level Talent Project (No. 825RC902), Key Project of Haikou Science and Technology Plan (No. 2024-007), and Joint Project of Hainan Provincial Health and Wellness Science and Technology Innovation (No. WSJK2025MS162).

Compliance with ethics guidelines

Conflicts of interest Chao Liang, Meihong Fu, Lunsha Deng, Yanping Zhuang, Chong Sun, Jiao Liu, Shaoping Chen, Hongjuan

Wang, Haimei Zhang, Zibao Huang, Li Yi, Kai Wang, and Ruibao Ren declare that they have no conflict of interest.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The trial's protocol received ethical approval from the hospital's Ethics Committee (Approval number: HKSZYYYLL-2023 (Ke)-10). Participants provided written informed consent during the screening process.

Electronic supplementary material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-025-1192-9 and is accessible for authorized users.

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