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Original Article

Folic acid supplementation improves cognitive function in participants with cerebral small vascular disease-related cognitive impairment: a randomized controlled trial

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ABSTRACT

Background: The potential improvement of cognitive function through folic acid (FA) supplementation in patients with vascular cognitive impairment (VCI) remains unclear, and no randomized controlled trials (RCTs) have been conducted specifically in populations with cerebral small vessel disease-related cognitive impairment (CSVD-CI).
Objective: This study aimed to explore the effects of FA supplementation on cognitive function and angiogenesis-related indicators in patients with CSVD-CI.
Design: Double-blinded, parallel group, randomized controlled trial, with a six-month follow-up period.
Setting: Department of neurology and neurosurgery in Shanxi, China.
Participants: 220 CSVD-CI patients.
Interventions: The intervention consisted of FA tablets (0.4 mg/tablet) administered orally at a dose of two tablets daily for six months, while the placebo tablets were identical in appearance and administration but lacked FA.
Measurements: The primary outcome was the Montreal Cognitive Assessment (MoCA) score at six months assessed in the intention-to-treat (ITT) population. Secondary outcomes included Mini-Mental State Examination (MMSE) score, Trail Making Test (TMT), Tinetti Performance Oriented Mobility Assessment (POMA), and five-level EuroQol five-dimensional questionnaire (EQ-5D-5 L).
Results: MoCA and MMSE scores improved significantly in the FA group compared to placebo (both $P < 0.05$). Additionally, the FA group had statistically significant increases in serum folate and decreases in serum homocysteine (Hcy) (both $P < 0.001$). Matrix metalloproteinase-9 (MMP-9) expression decreased significantly in the FA group compared with placebo ($P < 0.05$).
Conclusions: FA improved cognitive outcomes in CSVD-CI, accompanied by a reduction in serum Hcy levels and MMP-9 expression. Early FA supplementation could help prevent vascular-related cognitive decline in CSVD-CI patients.

1. Introduction

Cerebral small vessel disease (CSVD) is characterized by its impact on intracranial small vessels, including penetrating arterioles, capillaries, and venules, leading to brain tissue damage [1,2]. As the most common and progressive vascular disease, CSVD affects almost 70 % of the general population older than 60 years and nearly 100 % older than 90 years [3,4]. Notably, it is responsible for 45 % of dementia cases

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[5]. CSVD-related cognitive impairment (CI) is the most common cause of vascular cognitive impairment (VCI), ranging from mild vascular cognitive impairment (VaMCI) to vascular dementia (VaD), and is closely related to the risk of mortality [6,7]. Therefore, early intervention during the initial stages of CSVD-CI may help delay or prevent further cognitive deterioration and reduce the risk of long-term adverse outcomes.

Folic acid (FA), a water-soluble B-vitamin, plays crucial roles in nucleic acid metabolism and neural function regulation [8]. Although existing studies have confirmed the protective effects of FA intervention on cognitive function in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD), the cognitive benefits in VCI patients remain uncertain [9,10]. As of yet, among all the investigations in this field, only one trial has examined the effect of high-dose B vitamins (FA 5 mg/d, vitamin B_6 30 mg/d, and vitamin B_{12} 1 mg/d) on cognitive function and clinical efficacy in CSVD patients, observing improvements after just 4 weeks [11]. However, the effect of long-term, low-dose FA monotherapy on cognitive function in patients with CSVD-CI remains unknown.

Experimental and clinical evidence confirm that FA-mediated reduction of homocysteine (Hcy) effectively mitigates Hcy-induced endothelial dysfunction, a well-established contributor to vascular pathology progression [12,13]. Moreover, current observational studies have identified Hcy as an independent risk factor for CSVD-CI, with its levels showing a negative correlation with cognitive performance scores [14,15]. These findings suggest that FA supplementation could potentially improve cognitive performance in CSVD-CI by reducing elevated homocysteine levels, thereby attenuating Hcy-mediated neurovascular injury.

In this study, we aimed to investigate the effect of FA supplementation in 220 CSVD-CI patients. First, we hypothesized that daily supplementation with FA, compared with placebo, would result in greater improvement in global cognition over 6 months. Second, we examined the effects of FA supplementation on various metabolic and clinical outcomes. Third, we conducted a preliminary exploration of the impact of FA supplementation on angiogenesis-related parameters.

2. Methods

2.1. Study design

This randomized, double-blind, placebo-controlled trial was conducted at the Changzhi Institute of Traditional Chinese Medicine Affiliated Hospital (Changzhi, Shanxi province, China) from July 2024 to March 2025, evaluated the effects of FA consumption on cognitive and physical health in CSVD-CI. A 6-month intervention period was included. Participants visited the hospital at baseline (Month 0) and post-intervention (Month 6) for compliance checks. During each visit, blood samples were collected, and neuropsychological tests, motor function assessments, and quality-of-life evaluations were conducted. They were asked to maintain their usual diet, lifestyle habits, and medications during the intervention period, and to record any adverse events to ensure adherence to the study protocol.

The study was conducted in compliance with the ethical principles of the Declaration of Helsinki and was approved by the medical ethics committee of Tianjin Medical University, China. All participants provided signed informed consent. This work is registered on ChiCTR (Chinese Clinical Trail Registry; https://www.chictr.org.cn/) as ChiCTR2400085766.

2.2. Participants

Participants were recruited if they followed these criteria: (1) aged 40–80 years; (2) diagnosed with CSVD; (3) Mini-mental State Examination (MMSE, Chinese version) score $\leq$ 23 [16] or Montreal Cognitive Assessment (MoCA, Beijing version) score $\leq$ 26 [17]; (4) signed informed consent forms to participate in the trial. We excluded participants (1) with cognitive impairment due to depression, schizophrenia, bipolar disorder, psychotic disorder, toxicosis, or other systematic diseases; (2) with intracranial hemorrhage or significant bleeding from other organs; (3) with a history of new stroke within 3 months prior to admission; (4) with AD, Lewy body dementia, frontotemporal lobe dementia, brain tumors, hydrocephalus, or other central nervous system diseases; (5) with severe speech, visual or hearing disturbances that preclude cognitive evaluations; (6) inability to complete neuropsychological tests and magnetic resonance imaging (MRI) examinations; (7) participating in clinical trials of other interventions as subjects.

2.3. Diagnostic criteria of CSVD

The MRI markers of CSVD were defined according to the Neuroimaging Standards for Research into Small Vessel Disease [18]: (1) recent small subcortical infarct: axial imaging reveals infarcts with a diameter smaller than 20 mm, which may appear larger than 20 mm in coronal or sagittal views; (2) lacunes of presumed vascular origin: lacunes are round or ovoid lesions, 3–15 mm in diameter, located in subcortical regions, and exhibit cerebrospinal fluid; (3) white matter hyperintensity of presumed vascular origin: white matter hyperintensities are abnormal white matter signals of variable size, appearing hyperintense on T2-weighted or T2-FLAIR images; (4) perivascular space: perivascular spaces show signals identical to cerebrospinal fluid in all MRI sequences, appearing linear when the imaging plane is parallel to blood vessels and round or oval when perpendicular, typically measuring less than 3 mm in diameter; (5) cerebral microbleeds: cerebral microbleeds are identified as hypointense lesions on T2*-weighted gradient-echo MRI, reflecting magnetizing effects associated with small hemorrhages. Two experienced neurologists who were blinded to the participants' clinical information assessed all disease-related features based on MRI data. In cases of inconsistent assessments, a senior neurologist, who was unaware of the preliminary findings, made the final determination.

2.4. Sample size

Based on the previous study of FA intervention on cognitive function, the sample size was calculated to detect 1.92-point difference in MMSE scores between the two trial groups [19]. Accordingly, sample size of 88 per group would be sufficient to test difference between groups post-treatment assuming a 5 % significance, an 80 % power, and 4.5 standard deviations (SD). Considering the dropout rate of 20 %, each intervention group required at least 110 patients, resulting in a total sample size of 220.

2.5. Randomization and intervention

Patients were randomized in a 1 : 1 ratio to the FA group or the placebo group. The randomization sequence was created using SPSS 26.0 (IBM Corp., Armonk, NY, USA) and stored within sealed opaque envelopes. The main investigator in the center opened an envelope to obtain a number when a patient agreed to participate in the study. FA tablets were formulated as a daily oral dose of two tablets (Beijng Silian Pharmaceutical Co., Ltd., China; 0.4 mg/tablet; state medical permit No.: H10970079) for a duration of six months. The placebo tablets were identical to the FA tablets in appearance and composition, differing only by the absence of FA, with identical administration procedures between groups. Patients and investigators were blinded to the identity of the assigned treatment. Compliance was assessed by checking the medicine boxes and through telephone follow-ups.

2.6. Outcome assessments

The primary outcome was the MoCA score. MMSE score, Trail

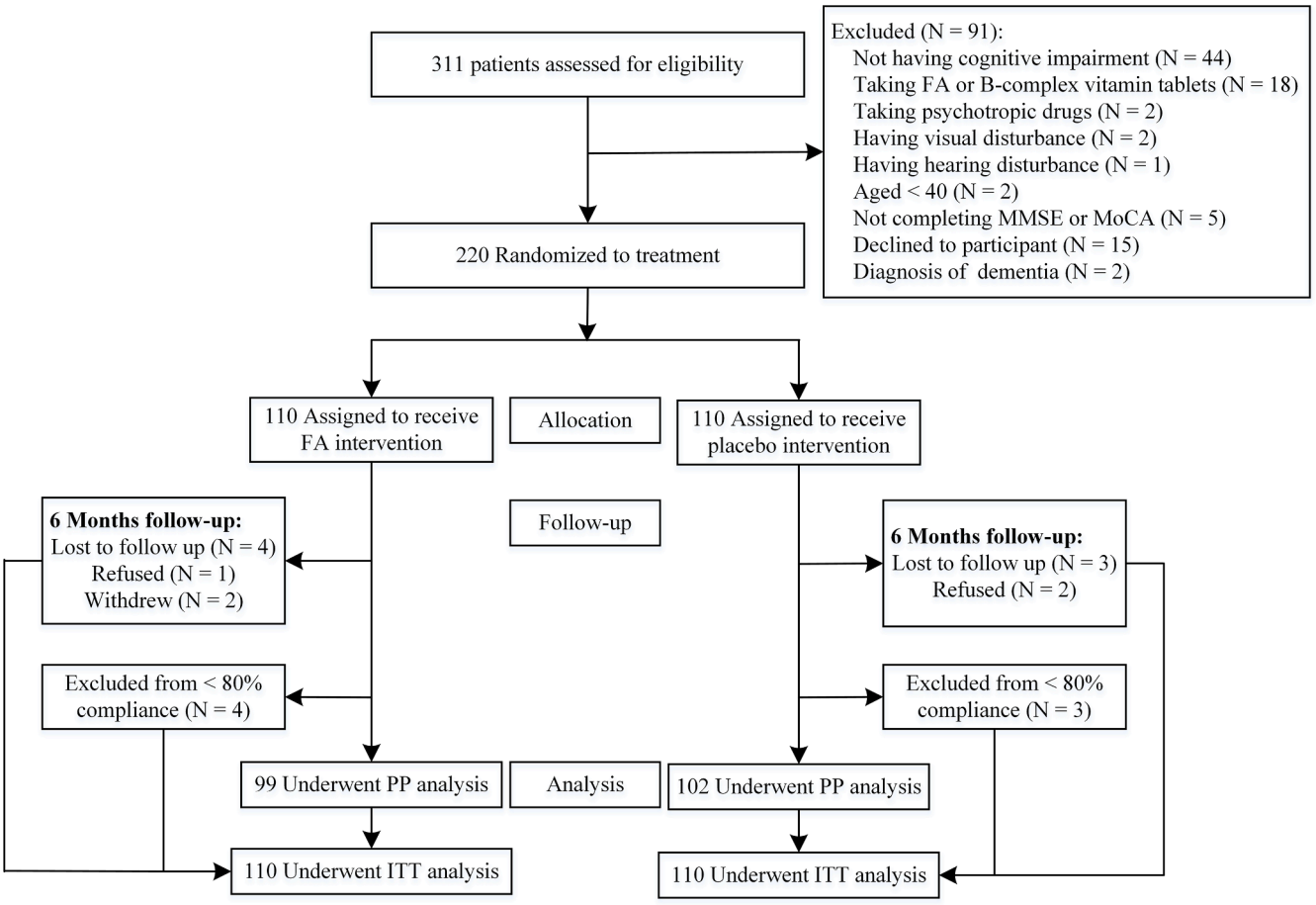

Fig. 1. Flow chart of study participants.
Abbreviations: FA, folic acid; ITT, intention-to-treat; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PP, per-protocol.

Making Test (TMT), Tinetti Performance Oriented Mobility Assessment (POMA), and five-level EuroQol five-dimensional questionnaire (EQ-5D-5 L) were secondary outcomes. These tests were conducted at baseline and 6-month follow-up.

The MoCA is a highly sensitive and specific cognitive screening tool used to distinguish between dementia and mild cognitive impairment with a total score of 30 points [20,21]. The higher scores indicate better cognitive function. MMSE is the most widely used tool for rapid cognitive screening in clinical and scientific research for general cognitive evaluation with a total score of 30 [22]. TMT consists of two parts, TMT-A and TMT-B, used to assess cognitive processing speed and executive function [23]. Tinetti POMA includes balance and gait tests to assess fall risk [24]. EQ-5D-5 L comprises an index score and a visual analog scale (VAS), which is used to estimate the quality of life [25,26].

2.7. Sample collection and clinical measurements

Twelve-hour fasting blood samples were collected at baseline and post intervention. Blood samples were collected into serum separating tube for serum and lithium heparin tube for plasma isolation and peripheral blood mononuclear cells (PBMCs). The concentration of serum folate and vitamin B_{12} were determined by the iFlash3000 automatic chemiluminescent immunoassay analyzer and its supporting kits (both manufactured by YHLO, Shenzhen, China). Meanwhile, the concentrations of Hcy, fasting glucose (FG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were analyzed by Beckman Coulter AU5800 (Beckman Coulter Inc., Brea, CA) and its supporting kits (Maccura Biotechnology Co., Ltd., Chengdu, China).

2.8. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of PBMCs was extracted by using SPARKeasy Frozen whole blood total RNA Kit (Sparkjade Biotechnology Co., Shandong, China) according to the manufacturer's protocol. The quality and concentration of the total RNA samples were determined by the Nanodrop 2000 spectrophotometer (Thermoscientific). For cDNA synthesis in RNA samples and genomic DNA elimination, SPARKscript II All-in-one RT SuperMix for qPCR (Sparkjade Biotechnology Co., Shandong, China) was used. mRNA expression levels were assessed by qRT-PCR on an Applied Biosystems QuantStudio 3 (Thermo Flasher Scientific, Waltham, MA, USA). The threshold cycle values of the 3 genes involved in angiogenesis-related indicators [matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), and vascular endothelial growth factor (VEGF)] were normalized to human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the endogenous control by using the $2^{-\Delta\Delta Ct}$ method. Primers are given in **Table S1**.

2.9. Sociodemographic and health-related characteristics

The baseline information questionnaire collected data on sex, age, height (m), weight (kg), education level ($\leq$ 12 years and $>$ 12 years), smoking status (current, former and never), drinking status (current, former and never), disease histories [hypertension, diabetes, hyperlipidemia and cardiovascular disease (CVD)], a food frequency questionnaire, activities of daily living (ADL) [27], self-rating anxiety scale (SAS) [28] and self-rating depression scale (SDS) [29]. These data were collected by trained investigators. Body mass index (BMI) (kg/m^2) was

Table 1
Baseline characteristics of all randomized participants[1].

Characteristics	Total (N = 220)	Placebo group (N = 110)	FA group (N = 110)	P
Age (years)	61.51±7.60	61.86±7.39	61.15±7.82	0.490
Sex, N (%)				0.058
Male	122 (55.5)	54 (49.1)	68 (61.8)	
Female	98 (44.5)	56 (50.9)	42 (38.2)	
BMI (kg/m^2)	25.29±3.09	25.12±3.07	25.31±3.12	0.417
Education level, N (%)				0.037
≤ 12 years	194 (88.2)	102 (92.7)	92 (83.6)	
> 12 years	26 (11.8)	8 (7.3)	18 (16.4)	
Smoking status, N (%)				0.301
Current	56 (25.5)	23 (20.9)	33 (28.0)	
Former	43 (19.5)	23 (20.9)	20 (21.5)	
Never	121 (55.0)	64 (58.2)	57 (51.8)	
Drinking status, N (%)				0.503
Current	35 (15.9)	15 (13.6)	20 (18.2)	
Former	43 (19.5)	20 (21.5)	23 (20.9)	
Never	142 (64.5)	75 (68.2)	67 (60.9)	
ADL (score)	14 (14, 15)	14 (14, 15)	14 (14, 16)	0.752
SDS (score)	26.25 (25.00, 30.00)	26.25 (25.00, 30.00)	27.50 (25.00, 31.25)	0.132
SAS (score)	25.00 (25.00, 28.75)	25.00 (25.00, 28.75)	25.63 (25.00, 28.75)	0.429
Folate intake (μg/d)	262.48 ±140.10	262.96 ±135.66	262.08 ±145.03	0.960
History of hypertension, N (%)	129 (58.6)	67 (60.9)	62 (56.4)	0.494
History of diabetes, N (%)	35 (15.9)	15 (13.6)	20 (18.2)	0.357
History of hyperlipidemia, N (%)	9 (4.1)	4 (3.6)	5 (4.5)	1.000
History of CVD, N (%)	27 (12.3)	12 (10.9)	15 (13.6)	0.538

Abbreviations: ADL, activities of daily living; BMI, body mass index; CVD, cardiovascular disease; FA, folic acid; IQR, interquartile range; SAS, self-rating anxiety scale; SD, standard deviation; SDS, self-rating depression scale.

[1] Normal data were presented as mean ± SD and compared using independent-samples t-test, while non-normal data were presented as median (IQR) and analyzed with Mann-Whitney U test. Categorical variables were expressed as N (%) and compared by the Chi-square or Fisher's exact test.

calculated as weight divided by the square of the height.

2.10. Statistical analysis

Normally distributed continuous variables were presented as mean ± standard deviation (SD) and were compared using independent-samples t-test between two groups and paired samples t-test within groups between baseline and post intervention, while non-normally distributed variables were presented as median [interquartile range (IQR)] and were compared using Mann-Whitney U test between two groups and paired samples Wilcoxon test within groups. Categorical variables are expressed as N (%), and differences are assessed using the Chi-square or Fisher's exact test.

The study was analyzed for the intention-to-treat (ITT) and per-protocol (PP) populations. By introducing the interaction term between the intervention groups and time, we carried out a mixed linear effect model to estimate the effect of the intervention on cognitive function, motor function, and quality of life. The models were adjusted for age, sex, BMI, education, smoking status, drinking status, history of diseases (hypertension, hyperlipidemia, diabetes and CVD), FA intake per day, ADL score, SDS score, and SAS score. The results are presented as β [95 % confidence interval (CI)]. To test the robustness of our findings, sensitivity analyses were performed with missing data handled by multiple imputation.

Data were analyzed using R (version 4.4.2) and figures were drawn using GraphPad Prism (version 8). P-value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

A total of 311 patients were screened for this study. Of these, 91 patients were excluded and the ITT analysis included all 220 patients. In addition, another 8 patients in the FA group and 5 patients in the placebo group were excluded after randomization (Fig. 1).

Baseline characteristics are presented in Table 1. There were 110 participants randomized to FA intervention group and 110 to placebo group. The mean (SD) age was 61.51 (7.60) years, females comprised 44.5 %, 88.2 % had lower than 13 years of education, 55.0 % reported never smoking, and 64.5 % reported never drinking. There was a significant difference in education levels at baseline between the FA and placebo groups. Other descriptive characteristics were comparable across intervention groups.

3.2. Changes in cognitive and physical health measures

Table 2 shows median (IQR) values and the results from the ITT and PP of intervention effects for cognitive and physical health measures at 6 months. Specifically, total MoCA score (ITT: β = 1.27; 95 % CI = 0.52, 2.03; PP: β = 1.15; 95 % CI = 0.37, 1.93) and total MMSE score (ITT: β = 0.76; 95 % CI = 0.14, 1.39; PP: β = 0.65; 95 % CI = 0.02, 1.28) were improved among patients with CSVD-CI in the FA intervention group compared with those in the placebo group at follow-ups after adjustment of potential confounders. However, participants randomized to FA intervention, compared with placebo, had no statistically significant benefit in TMT-A, TMT-B, total Tinetti POMA score, EQ-5D-5 L index score, EQ-5D-5 L VAS score, and other physical domains of MoCA and MMSE over 6 months.

Sensitivity analysis with imputation for participants lost to follow-up revealed a similar pattern of findings, namely, a statistically significant result in the comparison between the two groups with mixed models, wherein the FA intervention group was higher in total MoCA score and total MMSE score, but no significant difference in change in other cognitive tests or change in physical health measures within two-group comparison (**Table S2**).

3.3. Changes in clinical and laboratory results

Fig. 2 and **Table S3** presents the clinical and laboratory results of intervention effects at 6 months. There was a significant FA effect on serum folate levels (ITT: β = 6.39; 95 % CI = 4.55, 8.23; PP: β = 6.98; 95 % CI = 5.11, 8.85) and Hcy levels (ITT: β = −7.49; 95 % CI = −10.52, −4.46; PP: β = −7.85; 95 % CI = −11.00, −4.69). However, there was no significant effect on the remaining outcome parameters. After imputation of missing values, FA effects on serum folate level and Hcy levels remained stable (**Table S4**).

3.4. Changes in angiogenesis-related indicators

Changes in the relative expression of MMP-2, MMP-9, and VEGF at the mRNA level in two groups are shown in Table 3. In linear mixed models adjusted for potential confounders, there was a significant FA effect on MMP-9 expression in both ITT (β = −6.80; 95 % CI = −12.93, −0.64) and PP (β = −6.99; 95 % CI = −13.32, −0.64) analysis. However, no significant changes were observed in MMP-2 and VEGF expression, as well as in sensitive analysis (**Table S5**).

3.5. Compliance and adverse events

Of the 208 participants who completed the trial, adherence to the regimen was measured by having participants report the number of

Table 2
Baseline and post-intervention values for outcomes and results from linear mixed model analysis[1].

	Placebo group		FA group		ITT ($N = 220$)		PP ($N = 201$)	
	N	Median (IQR)	N	Median (IQR)	β (95 % CI)	*P*	β (95 % CI)	*P*
Cognitive function								
MoCA, score					1.27 (0.52, 2.03)	0.001	1.15 (0.37, 1.93)	0.004
Baseline	110	20 (18, 23)	21 (18, 23)					
Post-intervention	105	20 (18, 22)	21 (20, 23)					
MMSE, score					0.76 (0.14, 1.39)	0.019	0.65 (0.02, 1.28)	0.044
Baseline	110	26 (24, 27)	110	26 (24, 27)				
Post-intervention	105	26 (23, 27)	103	26 (25, 27)				
TMT-A test, second					−6.08 (−12.36, 0.06)	0.056	−4.85 (−11.28, 1.58)	0.141
Baseline	109	57.50 (44.76, 78.13)	109	60.40 (40.82, 76.58)				
Post-intervention	104	58.75 (46.69, 74.01)	102	54.33 (41.60, 66.87)				
TMT-B test, second					−1.73 (−14.69, 11.10)	0.793	−1.04 (−14.46, 12.35)	0.880
Baseline	107	100.66 (72.34, 151.13)	108	103.74 (74.31, 129.50)				
Post-intervention	103	99.91 (78.96, 145.2)	101	99.70 (80.21, 132.17)				
Motor function								
Tinetti POMA, score			−0.60 (−1.80, 0.60)	0.327		−0.63 (−1.88, 0.62)	0.328	
Baseline	110	27 (24, 28)	109	27 (22, 28)				
Post-intervention	104	27 (26, 27)	102	27 (26, 27)				
Quality of life								
EQ-5D-5 L index, score			0.01 (−0.01, 0.03)	0.309		0.01 (−0.01, 0.03)	0.309	
Baseline	110	0.95 (0.89, 1.00)	110	0.94 (0.90, 1.00)				
Post-intervention	105	1.00 (0.94, 1.00)	102	1.00 (0.95, 1.00)				
EQ-5D-5 L VAS, score			−3.01 (−7.17, 1.17)	0.159		−3.39 (−7.73, 0.96)	0.128	
Baseline	110	80 (75, 90)	110	82 (75, 90)				
Post-intervention	105	80 (80, 90)	102	80 (80, 90)				

Abbreviations: 95 % CI, 95 % confidence interval; ADL, activities of daily living; BMI, body mass index; CVD, cardiovascular disease; EQ-5D-5 L, five-level EuroQol five-dimensional questionnaire; ITT, intention-to-treat; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; POMA, Performance Oriented Mobility Assessment; PP, per-protocol; TMT, Trail-Making Test; VAS, visual analog scale.

[1] Linear mixed model was adjusted for sex, age, BMI, education level, smoking status, drinking status, disease histories (hypertension, diabetes, hyperlipidemia and CVD), dietary FA intake per day, ADL score, SAS score and SDS score.

remaining tablets and did not differ between groups. In the FA group, 7 participants discontinued compared to 5 in the placebo group. Additionally, 2 participants in the FA group withdrew due to gastrointestinal-related adverse events within one month of intervention initiation and a participant refused to comply with blood sampling at post-intervention in the placebo group. No other serious adverse events were reported in either group.

4. Discussion

To the best of our knowledge, this study represents the first RCT to evaluate the effects of low-dose FA supplementation on cognitive and physical health, metabolic indicators, and angiogenesis-related indicators in CSVD-CI patients. We found that daily supplementation with 0.8 mg of FA led to an elevation in serum folate levels, a reduction in serum Hcy levels, and a decrease in MMP-9 expression. Moreover, compared with the placebo group, the FA intervention improved global cognitive function as assessed by the MMSE and the MoCA.

Our study conducted a 6-month intervention with 0.8 mg/d of FA. Although current research lacked a consensus on the optimal dosage and duration for FA intervention, the following three aspects justify the rationale of our study. Firstly, a dosage of 0.8 mg/day over 6 months has been demonstrated to significantly reduce Hcy levels. A meta-analysis of 22 trials involving 3604 participants showed that interventions lasting ≤ 6 months can decrease Hcy levels by approximately 3.93 μmol/L [9]. Furthermore, Huang et al. [30] found that a stable dose-effect relationship was observed between 0 and 1.2 mg/day of FA therapy for Hcy reduction, but further increases in FA dose resulted in a plateau effect. Secondly, the dosage and duration in our study strike a balance between efficacy and safety, avoiding potential risks associated with long-term high-dose FA supplementation, such as masking vitamin B_{12} deficiency or causing neurotoxicity due to excessive intake [31,32]. Thirdly, low-dose FA supplementation has been shown to effectively prevent other diseases. A meta-analysis indicated that low-dose (≤ 0.8 mg/day) and relatively short-term (≤ 3 years) FA supplementation can reduce the risk of stroke by 23 % and 26 %, respectively [33]. Additionally, a study from the United States suggested that in regions without fortified grain, a supplementation of 0.8 mg/day falls within a safe range and avoids controversies linked to long-term high-dose use, such as the potential increased risk of certain cancers [34].

Our study demonstrated that FA intervention improved global cognitive function in patients with CSVD-CI, a subtype of VCI, aligning with some evidence. For instance, Kun MF et al. [35] found that 6 weeks of 3 mg/d FA supplement produced greater improvements in cognitive function compared to pre-intervention levels in poststroke patients with cognitive impairment (ΔMoCA = 4.06 ± 3.11). However, a meta-analysis in 2024 of 5 trials, examining intake of 0.5–15 mg/d of FA over 6 weeks-5.5 years, showed that cognitive function was not improved by a standardized mean difference of 0.16 (95 % CI = −0.28, 0.59) in VCI patients [9]. Specifically, several factors may account for

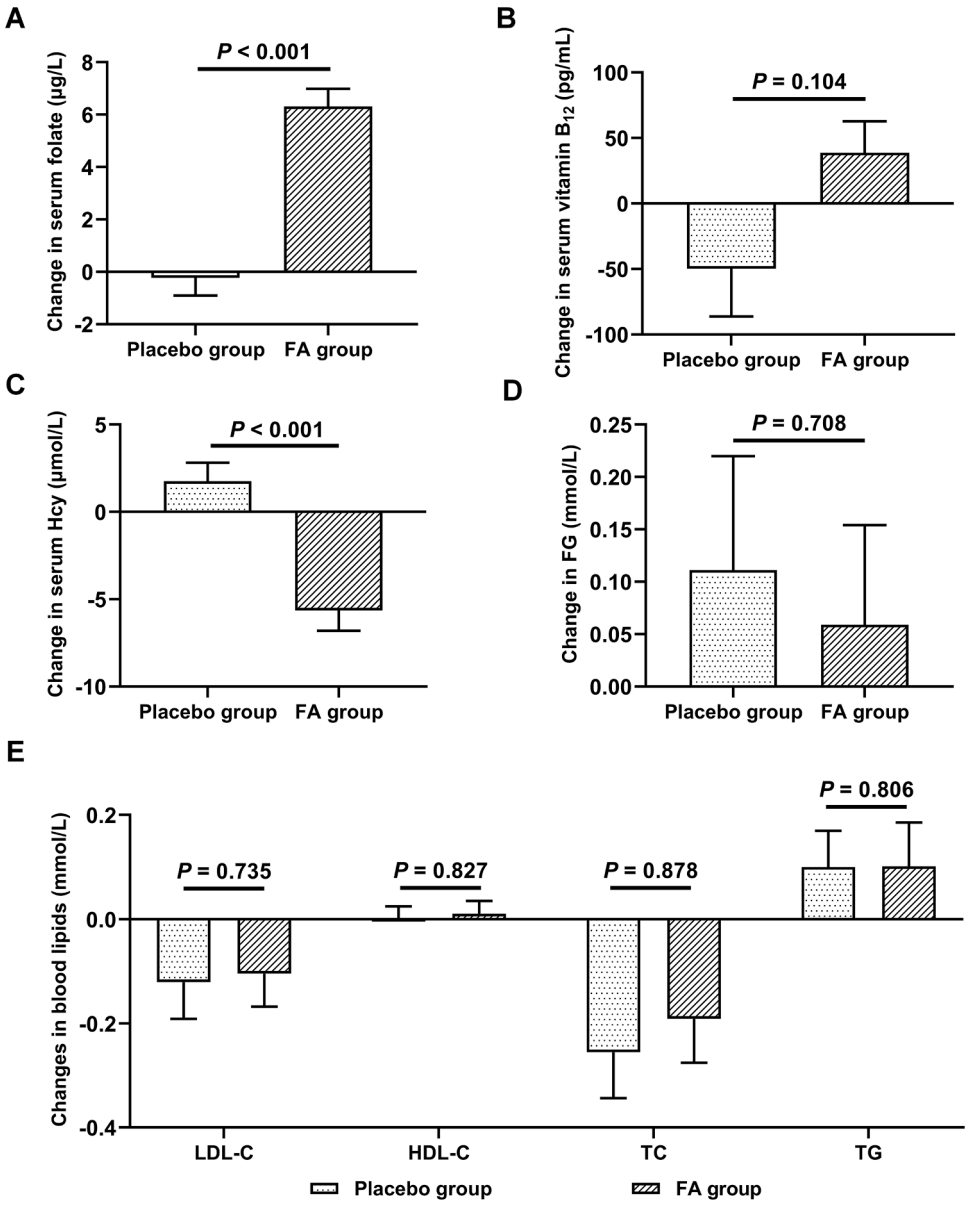

Fig. 2. Changes in clinical results of the placebo group and FA group at 6 months following intervention. Change between baseline and 6-month follow-up for levels of (A) serum folate (μg/L), (B) serum vitamin B_{12} (pg/mL), (C) serum Hcy (μmol/L), (D) fasting glucose (mmol/L), and (E) blood lipids (mmol/L), in the FA group and placebo group. Values are mean (SD), and *P*-values are shown for the group × time calculated from a linear mixed-effect model adjusted for sex, age, BMI, education level, smoking status, drinking status, disease histories (hypertension, diabetes, hyperlipidemia and CVD), dietary FA intake per day, ADL score, SAS score and SDS score.

Abbreviations: ADL, activities of daily living; BMI, body mass index; CVD, cardiovascular disease; FA, folic acid; FG, fasting glucose; Hcy, homocysteine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAS, self-rating anxiety scale; SDS, self-rating depression scale; TC, total cholesterol; TG, triglycerides.

the inconsistent findings. Firstly, the intervention in our study differed from previous research in both dosage and duration. We used a regimen of 0.8 mg per day FA over a period of six months. In contrast, the meta-analysis included studies with considerable variation in intervention doses and treatment lengths. These discrepancies may affect the outcomes. Secondly, research on VCI exhibits significant population heterogeneity. Existing studies predominantly focus on large-vessel stroke or AD with vascular damage, often overlooking small-vessel impairment such as that examined in our study. Additionally, the meta-analysis incorporated two European, one Oceanian, and two Asian studies, each from distinct countries. This diversity introduces genetic differences across ethnic groups, such as in MTHFR polymorphisms, which play a key role in folate metabolism and may influence responses to FA intervention [36]. Thirdly, pre-intervention folate status varies across study populations, which may influence intervention outcomes. Although baseline Hcy (Mean: 14.3–18.8 μmol/L) and folate (Mean: 17.4–21.7 nmol/L) levels in existing studies are generally comparable to ours, some studies used red blood cell folate measurements. Moreover, in regions with mandatory FA fortification, supplementation effects may be diminished due to higher baseline nutritional status, whereas greater benefits are observed in non-fortified areas such as Northern China, which is characterized by traditional diets low in leafy vegetables [37].

In the current study, we observed a decrease in Hcy levels after FA supplementation which is likely secondary to the one-carbon metabolism from FA. Folate serves as a crucial cofactor in the metabolism of Hcy, facilitating its conversion into methionine through methylation

Table 3
Baseline and post-intervention values for angiogenesis-related indicators and results from linear mixed model analysis[1].

	Placebo group		FA group		ITT ($N = 220$)		PP ($N = 201$)	
	N	Median (IQR)	N	Median (IQR)	β (95 % CI)	*P*	β (95 % CI)	*P*
MMP-2					−2.48 (−6.06, 1.09)	0.176	−2.30 (−5.91, 1.30)	0.212
Baseline	110	0.97 (0.25, 3.98)	110	0.91 (0.26, 6.86)				
Post-intervention	104	0.88 (0.21, 3.21)	103	0.64 (0.17, 2.97)				
MMP-9					−6.80 (−12.93, −0.64)	0.032	−6.99 (−13.32, −0.64)	0.033
Baseline	110	1.70 (0.38, 3.84)	110	1.63 (0.66, 5.41)				
Post-intervention	104	0.98 (0.25, 2.95)	103	1.23 (0.31, 3.18)				
VEGF					2.24 (−3.61, 8.08)	0.464	2.42 (−3.77, 8.61)	0.455
Baseline	110	1.12 (0.28, 3.37)	110	0.92 (0.36, 3.17)				
Post-intervention	104	0.85 (0.29, 2.57)	103	0.84 (0.16, 2.46)				

Abbreviations: 95 % CI, 95 % confidence interval; ADL, activities of daily living; BMI, body mass index; CVD, cardiovascular disease; FA, folic acid; IQR, interquartile range; ITT, intention-to-treat; MMP, matrix metalloproteinase; PP, per-protocol; SAS, self-rating anxiety scale; SDS, self-rating depression scale; VEGF, vascular endothelial growth factor.

[1] Linear mixed model was adjusted for sex, age, BMI, education level, smoking status, drinking status, disease histories (hypertension, diabetes, hyperlipidemia and CVD), dietary FA intake per day, ADL score, SAS score and SDS score.

reactions or cysteine through transsulfuration pathway, thereby reducing its toxic accumulation [38]. Supplementation with FA can effectively decrease plasma Hcy levels and ameliorate the oxidative stress induced by it, inflammation and cellular apoptosis, playing a regulatory role in cardiovascular diseases and neuroprotection [39,40].

Our study presents novel findings demonstrating that FA supplementation reduced the level of MMP-9 mRNA in CSVD-CI patients. Simultaneously, prior population-based studies, along with in vivo and in vitro experimental evidence, have also consistently established a significant association between FA and processes related to vascular remodeling and angiogenesis. For instance, an RCT with FA intervention in patients with epilepsy revealed that a 3-month supplementation of 5 mg/d FA significantly reduced carotid intima-media-thickness, flow-mediated dilation, and MMP-9 levels, while simultaneously improving the distensibility coefficient and β-stiffness index [41]. Similarly, FA treatment has been shown to mitigate the increased vascular MMP activity and downregulate the expression of MMP-2, MMP-9, and MMP-12 in mice with chronic hyperhomocysteinemia [42]. Additionally, FA has been demonstrated to modulate VEGF promoter methylation and subsequently inhibit its expression in ApoE knockout mice with atherosclerosis [43]. However, the discrepancies in MMP-2 and VEGF between our findings and existing evidence may be attributed to tissue-specific expression patterns and mRNA-protein expression disparity. The regulatory effects of FA may not be fully mirrored in peripheral blood analyses since circulating blood cells possess gene expression profiles distinct from brain vascular tissues. Furthermore, FA may directly modulate MMP activity and VEGF levels through multiple pathways, independent of transcriptional regulation. Future studies will be necessary to comprehensively explore the impact of FA intervention on vascular health in CSVD patients.

This study had several strengths, including inclusion of patients with CSVD; an in-person administration of cognitive and motor assessment; high follow-up and compliance rates; rich covariate data for addressing differences in treatment effects; and exploration of vascular health. However, several limitations should also be acknowledged. Firstly, although a balance was achieved among trial compliance, cost, and effect detection, the long-term consequences of FA intervention in patients with CSVD-CI remain unclear. Future studies could conduct long-term follow-up investigations to track cognitive decline, vascular events, and functional status for long-term FA intervention safety and efficacy. Secondly, due to sample size limitations, this study did not investigate whether the effects of FA intervention vary across different subtypes of CSVD patients. Future research should increase the sample size through multi-center collaboration to enroll diverse CSVD subtypes and conduct stratified analyses for subtype-specific intervention responses. Thirdly, our research was conducted at a single center, and potential dietary changes during the intervention period may have introduced confounding effects. Additionally, cognitive function assessments relied on subjective scales, which might lack objectivity compared to neuroimaging or biomarker-based evaluations. To address these issues, multicenter designs, combined with rigorous dietary monitoring (including weekly diaries, standardized guidance, and regular nutrient assessments) and the integration of neuroimaging, cognitive biomarkers, and traditional subjective scales, could strengthen generalizability, reduce bias, and enhance the objectivity of evaluations. Fourthly, our research preliminarily investigated the vascular-protective effects of FA intervention in CSVD-CI patients. The correlation between mRNA expression and protein activity remains uncertain, and there is a lack of direct evidence linking these peripheral blood biomarkers to brain tissue pathology. Clarifying these relationships requires in vitro verification of mRNA-protein correlations, paired peripheral blood and brain tissue sampling for biomarker-pathology links, and mechanistic studies on FA metabolism-related signaling pathways.

5. Conclusions

Our study provides the first prospective interventional evidence in patients with CSVD-CI, demonstrating improvements in cognitive function, Hcy levels, and MMP-9 mRNA expression following FA supplementation. The impact of FA supplementation on vascular health requires further validation in large-scale studies to confirm its protective role. Recommendations to supplement FA in those at CSVD-CI risk may assist in lowering AD risk through improvements in Hcy and vascular health, and may be a plausible and cost-effective strategy to tackle the burden of vascular-related cognitive decline.

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Ethics statement

This study involves human participants and was approved by the medical ethics committee of Tianjin Medical University on 3 April 2024 (No TMUhMEC20250005). Participants gave informed consent to participate in the study before taking part.

Data sharing statement

The data described in the manuscript will be made available upon request pending approval by the corresponding author.

Declaration of generative AI and AI-assisted technologies in the writing process

Generative AI and AI-assisted technologies were not used during the preparation of this manuscript.

CRediT authorship contribution statement

Yinyue Liu: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Zili Yu:** Investigation, Data curation. **Zhengjun Cai:** Validation. **Li Zhao:** Investigation. **Yu Wang:** Investigation. **Yajie Guo:** Investigation. **Xiaonan Su:** Software. **Yuli Miao:** Investigation. **Bin Yi:** Software. **Yanhong Wang:** Writing – review & editing, Writing – original draft, Resources, Project administration, Conceptualization. **Xumei Zhang:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors report no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tjpad.2025.100369.

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