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ORIGINAL ARTICLE

Folate therapy improves the stress-to-rest mean LV volume ratio in myocardial perfusion imaging in patients with diabetes

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Abstract
Objective Patients with diabetes have higher stress-to-rest mean left ventricular volume (SRLVV) ratio in myocardial perfusion imaging (MPI) and hyperhomocysteinemia. We studied the effect of folate therapy on SRLVV ratio and plasma homocysteine levels in patients with diabetes.
Methods Forty patients were enrolled and thirty-two completed the study. The patients underwent a 2-day pharmacological stress test and rest MPI before and 2 months after treatment with either 5 mg folic acid or placebo. SRLVV ratios were calculated, and plasma homocysteine levels were measured, before and after treatment.
Results Among the 32 patients who completed the study, 15 received folic acid and 17 received placebo. The age of subjects (folate 51.5 ± 6.1 years; placebo 50.6 ± 8.1 years), male/female ratio (folate 6/11; placebo 9/6),or MPI findings (proportion of normal results: folate 80.0 %; placebo 94.1 %) were similar between the two groups. The baseline SRLVV ratio was similar between groups (folate: 1.00 ± 0.09 vs. placebo: 0.97 ± 0.13); however, the post-treatment SRLVV ratio was significantly lower ($P < 0.001$) in the folate group than in the placebo group (folate: 0.93 ± 0.10 vs. placebo: 1.04 ± 0.17). A general linear repeated-measures model showed a significant difference in the change in SRLVV ratio between participants receiving folate and those receiving placebo. Post-treatment homocysteine level was lower after folate treatment (from 14.5 ± 4.6 to 11.5 ± 5.3 μmol/L), as compared to placebo (from 13.7 ± 5.0 to 17.9 ± 4.5 μmol/L) ($P = 0.01$). The changes in SRLVV ratio and homocysteine level were correlated ($r = 0.45$; $P = 0.016$).
Conclusions Short-term folate therapy reduced SRLVV ratio and plasma homocysteine level.

Keywords Diabetes · Endothelial dysfunction · Stress-to-rest mean left ventricular volume · Myocardial perfusion imaging · Folate · Homocysteinemia

Introduction

The prevalence and disease burden of diabetes is increasing globally [1] and in Iran [2]. One of the main underlying pathologies of diabetes is endothelial dysfunction (ED). ED results from hyperglycemia and the corresponding decreased activity or expression of nitric oxide synthase and enhanced superoxide production [3–5]. ED is associated with reversible myocardial perfusion defects in the absence of obstructive coronary artery disease, which could be ascribed to microvascular disease [6]. An abnormally high stress-to-rest mean left ventricular volume (SRLVV) ratio is also one of the stress-induced abnormalities in myocardial perfusion imaging (MPI) that may be unrelated to obstructive coronary artery disease [7]. High SRLVV ratio—transient ischemic dilatation (TID)—is a prognostic factor in cardiovascular disease in persons with diabetes mellitus [8] and correlates with difference in stress and rest

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LV ejection fractions [9], which is a risk factor for future cardiac events [10]. Considering the simultaneous correlation of ED and TID by abnormalities of MPI which may occur in the absence of obstructive coronary artery disease, we speculate ED may associate with TID through microvascular mechanisms [6, 7]. Abnormalities in methionine metabolism contribute to ED, via impaired nitric oxide production. Folate deficiency is a correctable contributory factor in such abnormalities [11]. In this randomized clinical trial, we examined the effect of folic acid supplementation on SRLVV ratio to provide evidence for the aforementioned hypothesis.

Materials and methods

Patients with diabetes were enrolled from two tertiary diabetes clinics: the private clinic of one of the authors (AE) and the diabetes clinic of Vali-Asr Hospital, Tehran University of Medical Sciences (Tehran, Iran). All diabetes diagnoses were confirmed by an endocrinologist. Patients were excluded if they had a history, or symptoms or signs, of coronary artery disease, including history of revascularization, coronary angiography, a positive exercise tolerance test, angina pectoris, and an abnormal electrocardiogram (both specific and nonspecific changes). This study was conducted in accordance with the Helsinki Declaration, and informed consent was collected from all patients. The ethics committee of Tehran University of Medical Sciences approved the study protocol.

The patients were referred to the department of nuclear medicine (Tehran University of Medical Sciences, Tehran, Iran) for MPI. Patients underwent a 2-day pharmacological stress and rest MPI at two centers: the Vali-Asr Hospital and Shariati Hospital. The stress study was performed with infusion of dipyridamole 0.14 mg/kg per min for 4 min, administration of 20 ± 2 mCi of ^{99m}Tc-sestamibi after a 4-min delay, and delayed imaging after 30–90 min. Rest phase imaging was done the next day, 45–120 min after injection of the same dose of tracer. Dual-headed gamma cameras at Vali-Asr Hospital (Forte ADAC; Philips, Milpitas, CA) and Shariati Hospital (Solus ADAC; Philips, Milpitas, CA) were used for ECG-gated SPECT cardiac imaging, with the following specifications: head position at 90 degrees, 64 × 64 matrix size, 180-degree data acquisition, 64 stops, and 30 ± 5 s per projection.

Patients were allocated by block randomization to the parallel treatment and placebo groups. The random sequence was generated by the assistant of the responsible endocrinologist (AE). The treatment and placebo groups received 5 mg/day folate or placebo, respectively, in a similar capsule for 60 days, after which MPI was repeated, using the same protocol. If an MPI was scheduled after day 60, patients were given additional medication. An overnight fasting blood sample was drawn from all participants on the day of the MPI. An ELISA kit (Pars Azmoon; Karaj, Iran) was used to measure plasma homocysteine levels. Because of logistic problems, eight patients underwent exercise tolerance testing instead of a pharmacological stress test for post-treatment MPI; data from these patients were thus excluded from the final analysis. Homocysteine data were not available for four patients, because of technical errors or nonparticipation in blood sampling. All researchers—including the referring endocrinologists, imaging specialists, coordinator, and data analyst—were blinded to treatment assignment throughout the study. There were no important deviations from the registered protocol for this randomized clinical trial (IRCT 2012120111624N1).

MPIs were interpreted by at least two nuclear physicians using visual assessment and evaluation of the QPS and QGS indices of AutoQUANT software (Cedars-Sinai, CA, USA). The scans were evaluated visually and with color scales in the AutoQUANT software. SRLVV ratio was automatically calculated by AutoQUANT software as the ratio of mean LV volume in the stress and rest phases [12]. Sum stress scores were also automatically generated by AutoQUANT, using a 5-point grading system (0: normal; 1: mildly decreased; 2: moderately decreased; 3: severely decreased; 4: absent) in a 20-segment model. The same grading system was used for visual assessment; scans showing mild ischemia of more than three segments or corresponding moderate or severe ischemia were considered abnormal.

Sample size was calculated to enable ascertainment of a change greater than 0.07 in SRLVV ratio at a P value of less than 0.05. The data were analyzed using SPSS Statistics version 17.0. General linear models (GLM) were designed for repeated measurements, to study changes in SRLVV ratio and homocysteine levels within and between groups before and after treatment. The paired t test and independent-sample t test were also used for comparisons when appropriate.

Results

Forty patients with diabetes participated in the study from July 2012 through June 2013: twenty in the placebo group and 20 in the treatment group. Eight patients (three in the treatment group and five in the placebo group) were excluded from the final analysis of change in SRLVV ratio, because they had received an exercise tolerance test instead of pharmacological stress test for the second MPI. The health characteristics of the participants were similar between the groups (Table 1). At baseline, 35 patients (87.5 %) had a normal MPI, four (10 %) had mild abnormalities, and one (2.5 %) had a moderate perfusion defect.

Table 1 Baseline health characteristics of the participants

	Placebo ($n = 17$; 9 males)	Folate ($n = 15$; 6 males)	Total ($n = 32$)	Sig
Age (years)	50.6 ± 8.1	51.5 ± 6.1	51.1 ± 6.9	0.7
BMI (kg/m^2)	28.2 ± 6.2	28 ± 3.7	28.1 ± 5.1	0.9
Waist circumference (cm)	90.5 ± 10.8	90.9 ± 10.1	90.7 ± 10.3	0.9
Duration of diabetes (years)	12.4 ± 5.5	12.5 ± 3.4	12.4 ± 4.6	0.9
HbA1c (%)	8.1 ± 1.5	8.6 ± 1.6	8.5 ± 1.5	0.4
Cholesterol (mg/dl)	206.22 ± 55.9	181.13 ± 31.87	189.82 ± 41.64	0.2
Triglycerides	183 ± 62.9	177.4 ± 57.4	182.5 ± 68.3	0.8
Creatinine	1 ± 0.1	0.9 ± 0.2	1 ± 0.1	0.6

The proportions were similar between groups (proportion of normal results: folate 80.0 %; placebo 94.1 %). Homocysteine levels were similar at baseline, then significantly decreased in patients receiving folic acid (from 14.5 ± 4.6 to 11.5 ± 5.3 μmol/L), but not in those receiving placebo (from 13.7 ± 5.0 to 17.9 ± 4.5 μmol/L). Table 2 shows MPI indices before and after treatment. SRLVV ratio was significantly lower after folate treatment (from 1.00 ± 0.09 to 0.93 ± 0.1; $P < 0.001$; Fig. 1), and the trend significantly differed between patients receiving folate and those receiving placebo ($F = 21$; eta = 0.57; observed power = 0.99; and $P < 0.001$). Change in SRLVV ratio was correlated with change in homocysteine level ($r = 0.45$ and $P = 0.016$).

Discussion

We found that short-term folate therapy corrected homocysteinemia in patients with diabetes and reduced SRLVV ratio. The decrease in homocysteine level suggests that folate therapy improves endothelial function in patients with diabetes. This is the first study to show that folate therapy has a beneficial effect on the MPI index. This finding highlights the therapeutic effect of folate supplementation on myocardial perfusion end points. The present concomitant decrease in SRLVV ratio and improvement in homocysteine level after folate therapy suggest a link between SRLVV ratio and ED.

Homocysteinemia contributes to ED via various mechanisms. Increased cellular homocysteine changes the structure of mitochondria and increases oxidant stress. Furthermore, nitric oxide is oxidatively inactivated and its production is reduced because of decreased mRNA transcription for glutathione peroxidase synthesis. Clinically, homocysteinemia results in endothelial swelling, vacuolization of endothelial cells, and increased fibrin deposition within arteries [13–15]. Homocysteine levels are higher in persons with diabetes [16, 17], mainly because of decreased renal function [18]. Some studies have reported that the microvascular complications of diabetes are associated with hyperhomocysteinemia [15, 16]; however, this association remains controversial [19–22]. Homocysteinemia is partially correctable with folate

Table 2 Myocardial perfusion indices before and after treatment; analyses within and between folate and placebo groups

	Placebo ($n = 17$)	Sig. level[a]	Folate ($n = 15$)	Sig. level[b]	Total ($n = 32$)	Sig. level[c]
$SRLVV_{baseline}$	0.97 ± 0.13	0.014	1.00 ± 0.09	<0.001	0.98 ± 0.11	<0.001
$SRLVV_{post\text{-}treatment}$	1.04 ± 0.17		0.93 ± 0.1		0.99 ± 0.15	
$SSS_{baseline}$	1.2 ± 1.3	0.265	1 ± 1.3	0.255	1.1 ± 1.3	NS
$SSS_{post\text{-}treatment}$	2.5 ± 4.8		1.6 ± 1.7		2.1 ± 3.7	
$EF_{baseline}$	64.7 ± 10.9	0.224	66.6 ± 14.2	0.892	65.6 ± 12.4	NS
$EF_{post\text{-}treatment}$	65.06 ± 10.81		66.73 ± 11.85		65.84 ± 11.16	
$ESV_{baseline}$	18.2 ± 5.6	0.063	20.1 ± 8.7	0.070	19.1 ± 7.1	NS
$ESV_{post\text{-}treatment}$	20.3 ± 6.5		19.8 ± 9.3		20.1 ± 7.8	
$EDV_{baseline}$	50 ± 11.1	0.905	53 ± 16.1	0.958	51.4 ± 13.5	NS
$EDV_{post\text{-}treatment}$	55.3 ± 12.5		59.2 ± 19.8		57.2 ± 16.3	

SRLVV ratio of stress mean LV volumes to rest mean LV volumes, *SSS* sum stress score, *EF* left ventricular ejection fraction, *ESV* end systolic volume, *EDV* end diastolic volume, *NS* non-significant

[a] Before and after treatment with placebo

[b] Before and after treatment with folic acid

[c] Between placebo and folic acid groups (repeated measurement general linear model adjusted for age and sex)

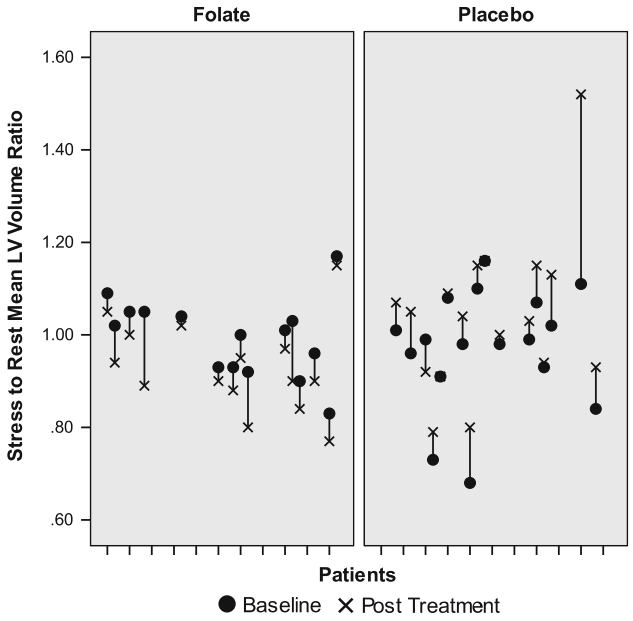

Fig. 1 Graphical depiction of the changes in the ratios of stress mean left ventricular volume to rest mean left ventricular volume (SRLVV) at baseline and after short-term treatment with acid folic or placebo

supplementation. Subsequent improvement in ED was confirmed in researchs examining changes in clinical indices, including albuminuria [23–26].

Many MPI indices are associated with myocardial perfusion abnormalities and coronary artery disease. SRLVV ratio is higher in persons with diabetes [27–30], particularly those who later develop cardiac events. Subendocardial ischemia or stress-induced stunning [31] may cause TID, which is an independent predictor of future cardiac events [8, 31]. We should note that the prevalence of ischemia was low in the study population, and most participants had a normal SRLVV ratio. Also, we did not collect or analyze data on mean LV volume at rest or during stress, because these variables were not printed in the output for our software. Such data might prove valuable in future studies. Also, post-treatment change of PET-measured responses of myocardial blood flow to cold pressor testing or vasodilator stress may provide further insight into the topic. Lastly, our finding indicating increase of the SRLVV ratio in subjects with diabetes without overt CAD in only 2 months is uncertain and needs further clarification in future studies.

Conclusion

Short-term folate supplementation lowered homocysteine levels in patients with type 2 diabetes. The improvement in SRLVV ratio among these patients suggests that this decrease substantially changed their cardiac risk profile.

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