ORIGINAL ARTICLE

The MTHFR C677T polymorphism influences the efficacy of folic acid supplementation on the nerve conduction studies in patients with diabetic polyneuropathy; A randomized, double blind, placebo-controlled study

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Background: Among patients with diabetic polyneuropathy, the status of folic acid, homocysteine, and nerve conduction studies (NCS) variations has been associated with methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms. The objective of the present study is to assess B9 vitamin supplementation associated with MTHRF C677T polymorphism can be effective on NCS variations in patients. **Materials and Methods:** This study is a randomized, double-blind, placebo-controlled study. Patients were randomly allocated to either intervention (1 mg of folic acid, $n = 40$) or placebo ($n = 40$) groups based on parallel group design. Blood samples were taken to determine the serum levels of folic acid and homocysteine. The NCS data were collected for the assessment of diabetic neuropathy. Genotyping was performed for C677T polymorphism of the MTHFR gene. **Results:** Four months after intervention, patients significantly observed change of serum folic acid and homocysteine levels based on C677T genotypes in the MTHFR gene. The amplitude of sensory peroneal nerve between intervention and placebo groups with CC genotype was significantly different (2.8 ± 1.6 vs. 1.9 ± 1.1). However, peak latency and amplitude of sensory sural nerve between CC (3.8 ± 1.8 vs. 4.0 ± 1.5 for peak latency and 3.5 ± 1.0 vs. 2.5 ± 1.0 for amplitude; and CT + TT genotypes (3.7 ± 1.7 vs. 3.9 ± 1.3 for peak latency and 3.2 ± 1.0 vs. 2.3 ± 1.1 for amplitude) were significant. Furthermore, significant difference for variables of motor tibial nerve and motor peroneal nerve amplitude was observed in different groups of MTHFR C677T genotypes (5.4 ± 2.9 vs. 4.6 ± 3.2 for onset-latency of tibial nerve between CC genotype; 4.8 ± 2.8 vs. 4.6 ± 3.2 for onset-latency of tibial nerve between CT + TT genotype; 0.6 ± 0.2 vs. 0.3 ± 0.1 for amplitude of tibial nerve between CC genotype; 0.5 ± 0.3 vs. 0.3 ± 0.2 for amplitude of tibial nerve between CT + TT genotype; 26.0 ± 13.3 vs. 23.2 ± 13.4 for velocity of tibial nerve between CC genotype; 26.0 ± 13.7 vs. 23.1 ± 9.6 for velocity of tibial nerve between CT + TT genotype; 1.6 ± 1.0 vs. 0.9 ± 0.7 for amplitude of peroneal nerve between CC genotype; 1.4 ± 0.7 vs. 0.9 ± 0.5 for amplitude of peroneal nerve between CT + TT genotype). **Conclusion:** The study determined that MTHFR C677T polymorphism effects the efficacy of folic acid supplementation on serum folic acid, homocysteine levels and some NCS parameters in diabetic polyneuropathy patients.

Key words: Diabetic polyneuropathy, folic acid, methylenetetrahydrofolate reductase

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INTRODUCTION

Diabetic neuropathy is a common complication among diabetes patients.[1] Factors including chronic hyperglycemia, diet, genetics,[2] lifestyle and other factors[3] are the causes of diabetic neuropathy. Reduced methylenetatrahydrofolate reductase (MTHFR) enzyme activity results in increased plasma homocysteine

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and reduced plasma folate.[4,5] Studies have determined a relationship between MTHFR polymorphism with vascular complications of diabetes including neuropathy.[6,7] No report has done to determine the influence of folate supplementation in related to MTHFR gene polymorphisms on the nerve conduction studies (NCS) variations in diabetic polyneuropathy. The aim of the present study was to assess if folate supplementation in associated with MTHFR C677T polymorphism can influence on the NCS parameters.

MATERIALS AND METHODS

Research design and sampling

This was a randomized, double-blind, placebo-controlled study. This research was approved by Isfahan University of Medical Sciences Ethics Committee (IRCT20121216011763N22). This research was done at Imam Mousa Sadr Clinic in Isfahan, Iran from May 22, 2017 to April 20, 2018. Inclusion criteria were patients with confirmed diabetic neuropathy by electromyography (EMG)-NCS, willingness to participate in the research and folate consumption during 16 weeks, not pregnant or planning to become pregnant. Nondiabetic neuropathic patients, users any supplement contain folate and Vitamin B12, consuming drugs of thyroid, methotrexate, bacterium, anticonvulsants, and sulfa antibiotics were excluded.

All patients completed the informed consent form. A total of 80 participants (mean age: 55 ± 5.8 years) were randomly allocated to either 1 mg of folic acid (intervention group, $n = 40$) or starch (placebo group, $n = 40$) during 16 weeks. Thirty-eight participants in the intervention group and thirty-seven participants in the placebo group completed the research. The linear regression performed to predict missing data after intervention with folate. At final, 40 patients in the intervention group and 40 patients in the placebo group analyzed [Figure 1]. Participants were told to not change their food consumption and physical activity.

Assessment of laboratory and diabetic neuropathy disease

Serum folic acid (normal range 3.9–16.9 ng/ml), Vitamin B12 (normal range 193–982 pg/ml) and homocysteine (normal range 5–15 micmol/l) levels were determined with automated immunoassay device (Immulite 2000 XPi Immunoassay System, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

The NCS variables for diabetic polyneuropathy assessment were done by EMG and nerve conduction velocity (EMG-NCS). Lower limb NCS was determined because lower limb nerves are involved more than upper limb nerves.[8]

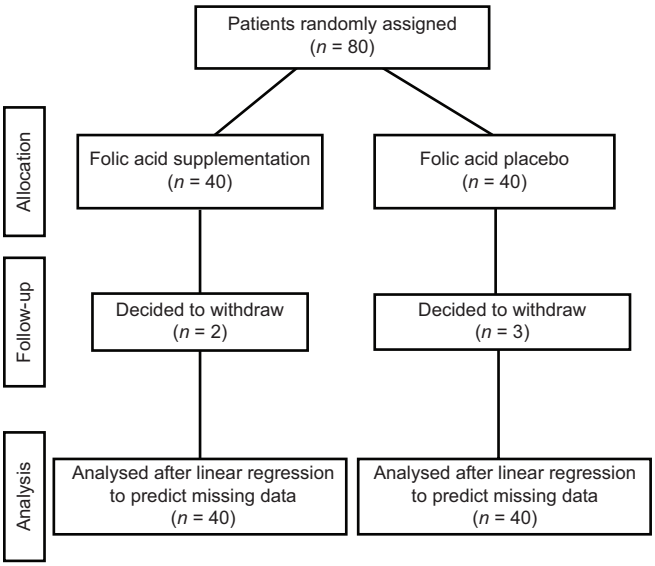

Figure 1: Flowchart patients

Genetic assessment

The MTHFR C677T genotypes were determined with polymerase chain reaction (PCR)-restriction fragment length polymorphism method.[9] Digestion of PCR products was performed using HinfI enzyme, and then agarose gel electrophoresis method used to separate DNA and observed using ultraviolet light. The HinfI enzyme digests the 198 bp band into 175 and 23 bp bands. The CC genotype results in the 198-bp fragment, whereas CT gives 198-bp and 175-bp bands, and TT generates a 175-bp band. The 23 bp band was not observed on agarose gel.[9]

Statistical analysis

Statistical analysis was conducted using IBM SPSS software (Inc. Chicago, IL, USA, version 20). P values < 0.05 were considered statistically significant. The assessment of data normality was performed using the Kolmogorov–Smirnov test. The serum levels of homocysteine had not normal distribution; therefore, we reported the log-transformed serum homocysteine levels in statistical analysis. Numerical variables were summarizes as mean ± standard deviation (SD) and categorical variables as the relative frequency in percentage (%).

Chi-square test and t-test were performed to assess comparison of categorical and continuous variables, respectively. The qualitative variables were expressed as frequency (percentage) and quantitative variables were expressed as mean (SD).

The means comparison before and after the intervention was assessed using paired t-test. The determination of differences between intervention and placebo groups with covariate variables adjustment was conducted by ANCOVA test.

RESULTS

Baseline characteristics of participants containing male sex, age, body mass index, waist circumference, and diabetes duration were similar between folic acid supplementation and placebo groups [Table 1]. The diabetes complications and comorbidities prevalence had not significant differences in intervention and placebo groups, as shown in Table 1. During the intervention with folate, daily food consumption reported unchanged, and intake of folic acid-fortified foods was not reported.

Patients were classified based on genotype to examine the effect of folic acid supplementation based on MTHFR C677T genotypes distribution. In the intervention group for MTHFR C677T polymorphism, 47.5% (n = 19) of patients the CC genotype, and 52.5% (n = 21) showed the CT/TT genotype. In the placebo group, 62.5% (n = 25) of patients showed the CC genotype and 37.5% (n = 15) showed the CT/TT genotype. 16 weeks after intervention with folate, patients significantly demonstrated the change of homocysteine (2.0 ± 0.2 vs. 2.1 ± 0.2 between CC genotype; 2.2 ± 0.1 vs. 2.3 ± 0.2 between CT + TT genotype) and folic acid levels. (11.0 ± 0.6 vs. 8.8 ± 0.8 between CC genotype; 9.3 ± 1.1 vs. 5.8 ± 1.3 between CT + TT genotype). Serum levels of vitamin B12 according to MTHFR C677T genotypes were not observed (490.4 ± 29.6 vs. 476.8 ± 43.2 between CC genotype, P = 0.595; 418.6 ± 53.3 vs. 346.6 ± 57.4 between CT + TT genotype, P = 0.130) [Table 2]. Decreased homocysteine was related to increase folic acid in intervention group [Table 2]. Supplementation with folate did not affect sensory peroneal peak-latency, onset-latency and velocity of motor peroneal nerve between genotypes groups of MTHFR C677T polymorphism [Tables 3 and 4]. Peroneal amplitude of sensory nerve was significant among patients with genotype CC (2.8 ± 1.6 vs. 1.9 ± 1.1; P = 0.043), but not CT + TT genotype in MTHFR gene [Table 3]. Regarding sensory and motor nerves variations of folic acid groups based on the MTHFR C677T genotypes, peak latency (3.8 ± 1.8 vs. 4.0 ± 1.5 between CC genotype; 3.7 ± 1.7 vs. 3.9 ± 1.3 between CT + TT genotype) and amplitude of sensory sural nerve (3.5 ± 1.0 vs. 2.5 ± 1.0 between CC genotype and 3.2 ± 1.0 vs. 2.3 ± 1.1 between CT + TT genotype), variables of motor tibial nerve (5.4 ± 2.9 vs. 4.6 ± 3.2 for onset-latency between CC genotype; 4.8 ± 2.8 vs. 4.6 ± 3.2 for onset-latency between CT + TT genotype; 0.6 ± 0.2 vs. 0.3 ± 0.1 for amplitude between CC genotype; 0.5 ± 0.3 vs. 0.3 ± 0.2 for amplitude between CT + TT genotype; 26.0 ± 13.3 vs. 23.2 ± 13.4 for velocity between CC genotype and 26.0 ± 13.7 vs. 23.1 ± 9.6 for velocity between CT + TT genotype) and motor peroneal nerve amplitude (1.6 ± 1.0 vs. 0.9 ± 0.7 between CC genotype and 1.4 ± 0.7 vs. 0.9 ± 0.5 between CT + TT genotype) were significant among MTHFR C677T genotypes (P < 0.05) [Tables 3 and 4]. In this assessment, decreased onset-latency and peak latency was observed in intervention group [Tables 3 and 4]. This decreased was associated with increased amplitude and velocity among patients [Tables 3 and 4]. The NCS variables did not reach normal values.

Table 1: Baseline characteristics of folic acid supplementation and placebo groups

	Folic acid (n=40)	Placebo group (n=40)	P
Male sex, n (%)	25 (62.5)	18 (45)	0.116*
Age, years	54.9±5.5	55.3±6.0	0.775**
BMI, kg/m^2	26.0±4.8	25.0±5.1	0.344**
Waist circumference, cm	96.7±4.9	97.5±3.6	0.428**
Diabetes duration (years)	12.4±3.2	11.9±3.4	0.464**
Diabetes complications and comorbidities, n (%)			
Retinopathy	10 (25)	14 (35)	0.329*
CHD	6 (15)	2 (5)	0.136*
Dyslipidemia	13 (32.5)	18 (45)	0.251*
Hypertension	26 (65)	21 (52.5)	0.256*

Data are shown as the mean±SD and n (%). *P-value was calculated by Chi-square test; **P-value was calculated by t-test. CHD=Coronary heart disease; SD=Standard deviation; BMI=Body mass index

DISCUSSION

The findings revealed that 1 mg folic acid supplementation per day for 16 weeks in diabetic peripheral neuropathy (DPN) patients significantly increased the serum levels of folic acid and reduced homocysteine among patients with MTHFR C677T polymorphism. Furthermore, 16 weeks intervention with folate supplementation increased sensory peroneal amplitude among patients with CC genotype, but not CT + TT genotype. Peak latency, the amplitude of sensory sural nerve, the amplitude of motor peroneal nerve, and variables of motor tibial significantly changed among patients with MTHFR C677T genotypes.

Previous studies have reported that folic acid deficiency is associated with increase the vascular brain and neurodegenerative diseases, and folate supplementation is related to increased development of nervous system and improves versus neurological diseases, including stroke, parkinson's, alzheimer's, depression, psychosis, and spinal cord injury.[10,11] Among the vitamins, folate can improve inflammation, oxidative damage, and insulin resistance in patients with diabetes. Related mechanism is the nerve growth factor expression improvement (nerve growth factor is essentially important in survival and differentiation neurons in the central nervous system), decreased malondialdehyde and free radicals levels. Folate supplementation as important antioxidant results in decreased free radicals and lipids peroxidation.[12] However, the mechanism of pathophysiological involvement in DPN is not known. The probable mechanism is increased

Table 2: Serum homocysteine, folate, Vitamin B12 levels in folic acid and placebo groups at baseline and after 16 weeks intervention based methylenetetrahydrofolate reductase C677T polymorphism

	Folic acid group (*n*=40)		Placebo group (*n*=40)		*P**	
	CC (19)	CT + TT (21)	CC (25)	CT + TT (15)	CC	CT + TT
Homocysteine (micmol/l)						
Baseline	2.1±0.2	2.3±0.1	2.1±0.1	2.3±0.1	-	-
After 16 weeks	2.0±0.2	2.2±0.1	2.1±0.2	2.3±0.2	<0.001[a]	0.003[a]
*P***	<0.001	<0.001	0.747	0.716	-	-
Folate (ng/ml)						
Baseline	9.0±0.6	7.4±1.0	8.9±0.7	5.9±1.0	-	-
After 16 weeks	11.0±0.6	9.3±1.1	8.8±0.8	5.8±1.3	<0.001[b]	<0.001[b]
*P***	<0.001	<0.001	0.273	0.579	-	-
Vitamin B12 (pg/ml)						
Baseline	489.6±28.0	416.6±53.8	476.8±43.5	349.7±55.6	-	-
After 16 weeks	490.4±29.6	418.6±53.3	476.8±43.2	346.6±57.4	0.595[c]	0.130[c]
*P***	0.474	0.151	>0.999	0.159	-	-

**P*-value was calculated by ANCOVA; ***P*-value was calculated by paired *t*-test; [a]Adjusted for waist circumference, hypertension, retinopathy and initial value; [b]Adjusted for sex, hypertension, CHD, dyslipidemia and initial value; [c]Adjusted for sex, hypertension, dyslipidemia, retinopathy and initial value. Results are demonstrated as mean±SD. Log-transformed levels of serum homocysteine were used. SD=Standard deviation; CHD=Coronary heart disease

Table 3: Nerve conduction study variations of sensory nerve in folic acid and placebo groups at baseline and after 16 weeks intervention based methylenetetrahydrofolate reductase C677T polymorphism

	Folic acid group (*n*=40)		Placebo group (*n*=40)		*P**	
	CC (19)	CT + TT (21)	CC (25)	CT + TT (15)	CC	CT + TT
Peroneal-nerve						
Peak-latency (msec)						
Baseline	4.6±2.1	3.9±1.6	4.0±1.5	4.0±1.7	-	-
After 16 weeks	4.0±2.1	3.8±1.9	4.0±1.4	4.2±1.9	0.476[a]	0.365[a]
*P***	0.004	0.571	0.948	0.278	-	-
Amplitude (μv)						
Baseline	2.6±1.6	2.8±1.8	2.2±1.0	2.2±0.8	-	-
After 16 weeks	2.8±1.6	3.3±1.7	1.9±1.1	2.2±0.9	0.043[b]	0.092[b]
*P***	0.107	0.001	>0.999	0.785	-	-
Sural-nerve						
Peak-latency (msec)						
Baseline	4.1±1.9	4.0±1.8	3.9±1.5	3.8±1.5	-	-
After 16 weeks	3.8±1.8	3.7±1.7	4.0±1.5	3.9±1.3	0.026[c]	0.019[c]
*P***	<0.001	0.015	0.184	0.436	-	-
Amplitude (μv)						
Baseline	2.9±0.7	2.9±0.9	2.6±0.8	2.6±0.8	-	-
After 16 weeks	3.5±1.0	3.2±1.0	2.5±1.0	2.3±1.1	<0.001[d]	0.012[d]
*P***	<0.001	0.003	0.291	0.240	-	-

**P*-value was calculated by ANCOVA; ***P*-value was calculated by paired *t*-test; [a]Adjusted for age, diabetes duration, CHD and initial value; [b]Adjusted for age, diabetes duration, retinopathy, CHD and initial value; [c]Adjusted for diabetes duration, CHD and initial value; [d]Adjusted for hypertension and initial value. Results are demonstrated as mean±SD. Log-transformed levels of serum homocysteine were used. CHD=Coronary heart disease; SD=Standard deviation

levels of homocysteine or oxidative stress for DPN development.[12] It is reported that folate is related to increased homocysteine remethylation.[12] Therefore, we assessed serum homocysteine levels and an increased serum level of homocysteine was observed.

The animal study was demonstrated that folate among rats with diabetes could be protective versus DPN disease.[12] The any human research performed to assess the effect folic acid supplementation based on MTHFR gene polymorphisms on NCS variables among patients with DPN. Association analysis using DNA polymorphisms will help to show the peoples genetic traits such as disease risk, drug response, and aging. Recent studies have demonstrated special notice for relationship between single-nucleotide polymorphisms and diseases risk.[13] Studies have demonstrated that MTHFR C677T genotypes are related to increase vascular problems, including diabetic retinopathy, nephropathy,[14-16] and DPN risk;[17] however, research performed by Kaye *et al*. shown no relationship between MTHFR C677T polymorphism and vascular problems of type 2 diabetes.[8] In 2012, the systematic review research demonstrated that MTHFR gene C677T polymorphism was significantly related to diabetic nephropathy and diabetic retinopathy.[9] The

Table 4: Nerve conduction study variations of motor nerve in folic acid and placebo groups at baseline and after 16 weeks intervention based methylenetetrahydrofolate reductase C677T polymorphism

	Folic acid group (*n*=40)		Placebo group (*n*=40)		*P**	
	CC (19)	CT + TT (21)	CC (25)	CT + TT (15)	CC	CT + TT
Peroneal-nerve						
Onset-latency (msec)						
Baseline	5.8±1.3	5.7±0.8	5.1±1.6	4.9±1.5	-	-
After 16 weeks	5.6±1.3	5.2±1.1	5.4±1.8	4.8±1.5	0.116[a]	0.052[a]
*P***	<0.001	0.023	0.152	0.207	-	-
Amplitude (mv)						
Baseline	1.1±0.9	1.1±0.6	1.0±0.7	1.0±0.7	-	-
After 16 weeks	1.6±1.0	1.4±0.7	0.9±0.7	0.9±0.5	<0.001[b]	0.010[b]
*P***	<0.001	0.006	0.040	0.628	-	-
Velocity (m/sec)						
Baseline	28.1±8.8	30.3±5.8	30.1±7.6	29.3±7.2	-	-
After 16 weeks	29.2±8.8	31.2±5.7	30.2±7.4	29.5±7.2	0.234[c]	0.074[c]
*P***	0.004	0.003	0.656	0.297	-	-
Tibial-nerve						
Onset-latency (msec)						
Baseline	6.0±3.2	5.1±2.9	4.5±3.1	4.5±3.1	-	-
After 16 weeks	5.4±2.9	4.8±2.8	4.6±3.2	4.6±3.2	0.019[d]	0.020[d]
*P***	0.032	0.015	0.230	0.188	-	-
Amplitude (mv)						
Baseline	0.3±0.2	0.4±0.2	0.3±0.2	0.4±0.2	-	-
After 16 weeks	0.6±0.2	0.5±0.3	0.3±0.1	0.3±0.2	<0.001[e]	0.004[e]
*P***	0.001	0.001	0.306	0.300	-	-
Velocity (m/sec)						
Baseline	24.7±13.3	24.5±13.7	23.1±13.6	24.1±9.8	-	-
After 16 weeks	26.0±13.3	26.0±13.7	23.2±13.4	23.1±9.6	<0.001[f]	<0.001[f]
*P***	<0.001	<0.001	0.965	0.167	-	-

*,***P* values < 0.05 were considered statistically significant. [a]Adjusted for age, BMI, diabetes duration, retinopathy, hypertension, and initial value; [b]Adjusted for waist circumference and initial value; [c]Adjusted for sex, waist circumference, dyslipidemia, and initial value; [d]Adjusted for diabetes duration and initial value; [e]Adjusted for sex, BMI, CHD and initial value; [f]Adjusted for age, waist circumference and initial value. CHD=Coronary heart disease; BMI=Body mass index

retinopathy associated with MTHFR C677T mutation found in DPN patients.[17] Reduced MTHFR enzyme activity lead to increased plasma levels of homocysteine and reduced folate.[4,5] The plasma homocysteine levels were evaluated based on MTHFR A1298C and C677T polymorphisms in type 2 diabetic patients. The results showed that individuals with A1298C polymorphism had lower levels of homocysteine than C677T polymorphism in MTHFR gene.[10] Compared with the A1298C polymorphism, individuals with C677T polymorphism in MTHFR gene had a greater reduction in MTHFR activity and lower levels of folic acid plasma and increased plasma homocysteine.[11,14] The studies showed that MTHFR A1298C polymorphism along with other factors may be associated with increased homocysteine.[11,18] The present results about effect of folic acid supplementation on serum levels of homocysteine, folic acid and NCS variation may be due to the influence of MTHFR polymorphisms that may change the response to folic acid supplementation. The MTHFR enzyme gene variants have been associated with different response to folic acid supplementation.[11,14] Thus, we assessed the influence of folic acid supplementation based on genotype distribution of the MTHFR C677T polymorphism. The levels folic acid and homocysteine serum had significant difference between MTHFR C677T polymorphism. In addition to the effect of the folic acid supplementation, genotype of patients with MTHFR C677T polymorphism can be effective on NCS variations. However, variables did not each to normal value. In this assessment, motor peroneal amplitude before and after study in placebo group among patients with CC genotype was significant. Therefore, the finding of NCS can be influenced by other conditions, including skin temperature, height, and examiner. Reduced skin temperature was related to lower velocity. Furthermore, increased height was related to lower velocity and amplitude.[16]

Our research had strengths and limitations. First, the present reports are the first clinical trial study to demonstrate the finding that the folate supplementation based on MTHFR C677T polymorphisms affects the NCS variations among patients with diabetic polyneuropathy. Second, in this research, covariates have been controlled in the results of research. Furthermore, this research had limitation. It is suggested that covariates such as skin temperature be controlled in the finding ofresearch. Because, the results

of NCS can be affected by variables, including skin temperature. Second, other MTHFR polymorphisms such as A1298C and epigenetic modification are assessed in future studies.

CONCLUSIONS

In summary, data from the present study revealed that patients with MTHFR C677T genotypes had beneficial results on the NCS. Due to the useful influences of the MTHFR C677T polymorphism on the response to folic acid, more long-term studies with larger number of sample sizes based on polymorphisms of MTHFR gene and different genotypes should be performed to demonstrate the genetic and NCS interactions after intervention with folic acid among DPN patients.

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Conflicts of interest

There are no conflicts of interest.

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