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The effects of folic acid and pyridoxine supplementation on characteristics of migraine attacks in migraine patients with aura: A double-blind, randomized placebo-controlled, clinical trial

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ABSTRACT

Objective: The aim of this study was to assess the effects of folic acid alone and in combination with pyridoxine on characteristics of migraine attacks in adult migraine patients with aura.
Methods: This double-blind, randomized placebo-controlled, clinical trial was conducted on 95 migraine patients with aura (age range 18–65 y) in Isfahan, Islamic Republic of Iran, in 2014. Patients were randomly allocated to receive folic acid (5 mg/d) plus pyridoxine (80 mg/d) or folic acid alone (5 mg/d) or placebo (lactose) for 3 mo. Characteristics of migraine attacks including headache severity, attacks frequency, duration, and headache diary results (HDRs) were obtained for each patient at baseline and at the end of the study.
Results: Folic acid plus pyridoxine intake resulted in a significant decrease compared with placebo in headache severity (-2.71 ± 0.08 versus -2.19 ± 0.05; $P < 0.001$), attack frequency (-3.35 ± 0.09 versus -2.73 ± 0.05; $P < 0.001$), duration (-7.25 ± 0.17 versus -6.5 ± 0.07; $P < 0.001$), and HDR (-74.15 ± 0.2 versus -72.73 ± 0.1; $P < 0.001$). Additionally, the reduction in these characteristics of migraine attacks in the folic acid plus pyridoxine group was significant compared with the group given folic acid alone ($P < 0.001$). However, these beneficial effects of the combined supplement became nonsignificant for attack duration compared with the folic acid–only and placebo groups after controlling for confounders. Folic acid intake without pyridoxine did not lead to a significant decrease in characteristics of migraine attacks compared with placebo group.
Conclusions: Supplementation of folic acid with pyridoxine could decrease the characteristics of migraine attacks including headache severity, attack frequency, and HDR; however, further studies are needed to shed light on the findings of the present study.

Introduction

Migraine is an intermittent neurovascular headache disorder that usually is characterized by recurrent headache, nausea, vomiting, sensitivity to light and sound, neck pain, muscle tension, photophobia, and phonophobia [1]. Migraine headaches are typically one-sided and throbbing. They last between 4 and 72 h [2]. This disorder is most prevalent in middle-aged individuals

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and women [3]. The prevalence of migraine in Iranian adults is reported from 7.14% to 18.11% [4]. Based on International Headache Society (IHS) criteria, there are two major classes of migraine: migraine with aura (MA) and migraine without aura. These two subtypes have, to some extent, the same symptoms; however, 25% of patients with migraine perceive an aura, which is a transient disturbance in visual, sensory, language, or motor function and defined as a signal of headache occurrence [5].

Migraine headaches result in a substantial reduction in quality of life and have heavy costs for migraine sufferers [6]. Various drugs used to reduce migraine symptoms and frequency of migraine attacks often are expensive and have many side effects [7]. It has been shown that some nonpharmacologic therapies such as relaxation training, butterbur, vitamin D, riboflavin, magnesium, and coenzyme Q10 supplementation are effective for improving migraine symptoms [8–11]. Recently, it was reported that folic acid and pyridoxine supplementation can lessen migraine symptoms by affecting homocysteine levels [12,13]. In one study, folic acid and pyridoxine intake in combination with cobalamin decreased the severity and frequency of migraine attacks [12]; however, another study found that intake of these vitamins reduced the migraine severity but had no effects on frequency of attacks [13]. Earlier studies have mostly focused on combination effects of folic acid and pyridoxine but single effects of these vitamins have not been studied as frequently. Moreover, prior studies mostly have been confined to Western nations and data in this regard are scarce in Asian countries. Therefore, this study aimed to assess the effects of folic acid alone and in combination with pyridoxine on characteristics of migraine attacks in Iranian migraine patients.

Material and methods

Study design

This was a parallel, double-blind, randomized placebo-controlled trial with all those involved—investigator, patients, and the researcher who delivered the drug—being blinded to the therapeutic option. The trial was registered in the Iranian Registry of Clinical Trials.

The trial was approved by the Institutional Review Board and Ethics Committee of Isfahan University of Medical Sciences (Isfahan, Iran). This study followed declarations of Helsinki, and written consent was obtained from all patients, and verbal explanation about the research and assurance of confidentiality and anonymity was provided before the enrollment.

Participants

This study was performed in Khorshid and Emam Mosa Sadr clinics of Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran, from January 8 through April 16, 2014. The study included individuals who were referred to recruitment centers who were between the ages of 18 and 65 y; had history of migraine for ›5 y; had a 1-y history of severe, recurrent, and long-lasting migraine attacks (at least one attack per mo lasting 4 h); had a current diagnosis of MA approved by an experienced neurologist according to IHS (third revision) beta diagnostic criteria [5]; and were receiving routine treatment of migraine. We selected patients with MA based on previous studies [14,15] reporting that homocysteine levels are high in these patients. *Migraine attack* was defined as one-sided headache with aura in each situation in a day. Individuals were excluded if they were taking vitamin supplements or had clinical cardiovascular diseases, previous stroke, and chronic renal failure, which may increase homocysteine levels.

Sample size was determined by formula suggested for randomized clinical trials, with type I error of 5%, type II error of 20%, study power of 80%, and serum level of homocysteine as a key variable. The number of needed samples was calculated as 30 participants. To get a more confident result with a 20% dropout rate, we considered 34 patients in each group.

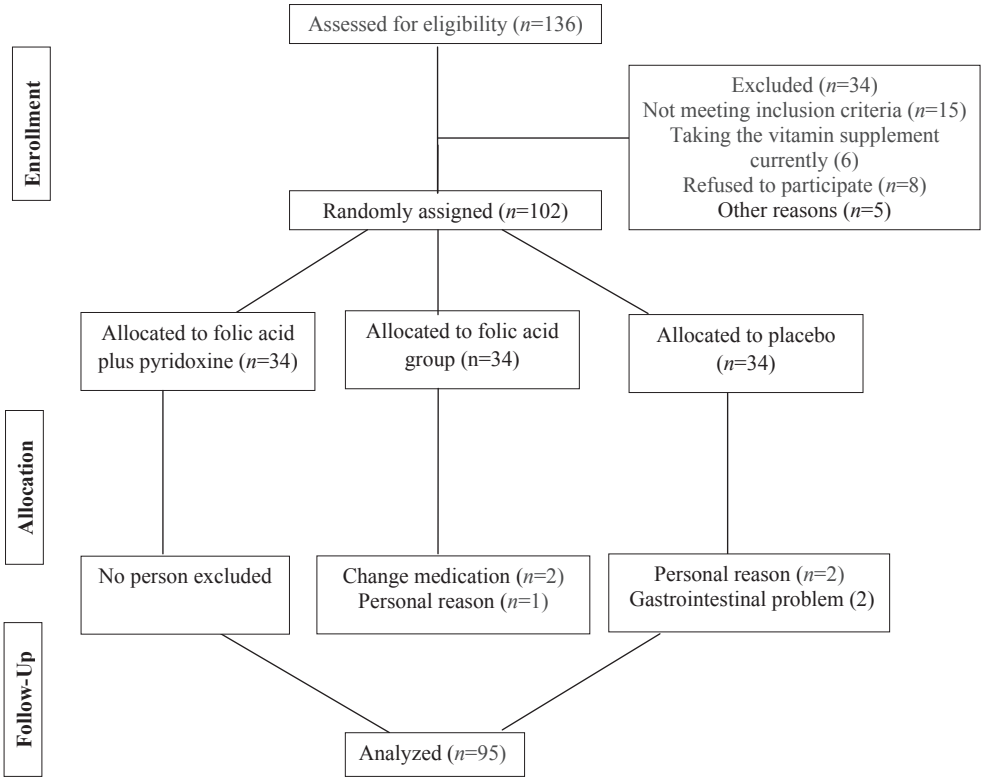

Fig. 1. Summary of patient follow-up.

Table 1
Baseline characteristics of migraine patients in three intervention groups

Variables	Folic acid + pyridoxine (n = 34)*	Folic acid (n = 31)†	Placebo (n = 30)‡	*P* value§
Sex (female %)	73.21	71.32	80.80	0.6
Age (y)	33.54 ± 1.6	37.38 ± 1.92	35 ± 2.12	0.35
BMI (kg/m^2)	26.49 ± 0.89	25.42 ± 0.64	25.73 ± 0.98	0.3
Hcy (μm/L)	9.12 ± 0.57	8.16 ± 0.49	8.01 ± 0.58	0.43
Severity (VAS)‖	8.93 ± 0.02	9 ± 0.01	8.98 ± 0.02	0.09
Frequency (per mo)	12.14 ± 0.11	12.41 ± 0.16	12.37 ± 0.18	0.38
Duration (h)	22.78 ± 0.15	22.77 ± 0.15	22.69 ± 0.15	0.92
HDR¶	193.40 ± 0.18	193.66 ± 0.25	193.55 ± 0.23	0.69
Family history of migraine (%)	56.81	54.82	57.70	0.24

BMI, body mass index; HDR, headache diary result; Hcy, homocysteine; VAS, visual analog scale
All values are presented as mean (SE) or percent
* Received daily capsule containing 5 mg folic acid plus 80 mg pyridoxine.
† Received daily capsule containing 5 mg folic acid.
‡ Received daily capsule containing lactose.
§ Obtained from analysis of variance.
‖ Measured by VAS that ranked headache severity from 1 to 10.
¶ Determined by formula: frequency × duration.

Randomization

Patients were randomly allocated to three groups of folic acid plus pyridoxine (n = 34), folic acid alone (n = 34), or placebo (n = 34) based on age, sex, and body mass index (BMI) using a computer-generated list of random numbers by an individual outside the study, which was concealed by the use of security envelopes.

Assessment of variables

We used a researcher-made checklist to collect information about age, weight, height, BMI, medical history, medication, and supplement use at the beginning of study. Height was measured in a standing position without shoes using a tape measure with the nearest 0.5 cm. Weight was determined with minimal clothing on and without shoes by analog scale with a precision of 100 g. BMI was calculated as weight in kilograms divided by height in square meters (kg/m^2). Blood samples (10 mL) were collected at baseline in the early morning after an overnight fast. Serum homocysteine levels were analyzed using enzyme-linked immunosorbent assay method (Liquid Stable 2-part homocysteine reagent kit: Roche Company, Germany). Based on this method, *hyperhomocysteinemia* is defined as serum levels >15 μmol/L in men and >10 μmol/L in women [16]. Because of normal values of homocysteine at the baseline, we did not measure the homocysteine level at the end of the trial. We determined characteristics of migraine attacks including severity, frequency, duration (h), and headache daily results (HDRs) at the baseline and end of the study. To measure severity, we used visual analog scale (VAS) ranking the severity of headache from 1 to 10 [17]. Based on this method, patients were asked to score headaches that occurred in the previous month, from 1 to 10. The number of migraine attacks in a month was considered as frequency of migraine attacks. To determine the mean duration of migraine attacks per day, named HDR, we used the following formula: frequency of attacks × duration of headache [17]. The incidence of adverse events (AEs) was evaluated by recording all observed or volunteered AEs. For this purpose, any study-related AEs during treatment were monitored by weekly evaluation. For patients who withdrew or those lost to follow-up, AEs were acquired by telephone.

Table 2
Dietary intakes of migraine patients in the three intervention groups

Variables	Folic acid + pyridoxine (n = 34)*	Folic acid (n = 31)†	Placebo (n = 30)‡	*P* value§
Energy (kcal/d)	1948.07 ± 125.4	2736.45 ± 163.3	2477.31 ± 141.5	<0.001
Protein (g/d)	60.34 ± 3.83	86.88 ± 6.38	76.07 ± 4.15	0.001
CHO (g/d)	324.42 ± 20.95	415.75 ± 23.38	406.24 ± 25.19	0.007
Fat (g/d)	49.86 ± 4.22	87.6 ± 8.18	67.93 ± 6.33	<0.001
Riboflavin (mg/d)	1.58 ± 0.06	1.58 ± 0.07	1.44 ± 0.08	0.28
Pyridoxine (mg/d)	1.23 ± 0.05	1.39 ± 0.1	1.28 ± 0.09	0.24
Folate (μg/d)	231.71 ± 11.31	285.69 ± 21.29	243.81 ± 20.98	0.1
Cobalamin (μg/d)	3.02 ± 0.17	3.8 ± 0.46	2.78 ± 0.36	0.15
Magnesium (mg/d)	222.42 ± 7.52	247.35 ± 14.48	244.62 ± 21.28	0.3
Sodium (g/d)	7.6 ± 0.6	8.4 ± 0.7	8.4 ± 0.8	0.7
SFAs (g/d)	18.7 ± 0.93	19.4 ± 1.24	17.5 ± 1.35	0.13
MUFAs (g/d)	16.72 ± 0.69	20.6 ± 1.46	16.41 ± 1.27	0.005
PUFAs (g/d)	16.67 ± 0.88	20.84 ± 2.62	18.05 ± 2.25	0.12
Linoleic acid (g/d)	16.3 ± 1.1	19.8 ± 2.6	17.1 ± 2	0.49
Linolenic acid (g/d)	0.25 ± 0.02	0.29 ± 0.04	0.3 ± 0.04	0.73
ω-6/3	84 ± 18.5	70.5 ± 8	63.6 ± 8.6	0.48
Food groups (g/d)				
Fruits	274.9 ± 40.9	290.8 ± 28.5	301.9 ± 32.3	0.85
Dairy	127.4 ± 22.2	75.6 ± 13.2	52.6 ± 12	0.006
Nuts	51 ± 9.5	70.8 ± 10.6	76.4 ± 9.7	0.2
Tomato	49 ± 12.7	9.2 ± 2.1	8.6 ± 2.9	<0.001
Chocolate	0.56 ± 0.3	0.06 ± 0.05	0.001 ± 0.06	0.03
Citrus	57 ± 15.3	30.8 ± 4.7	31.1 ± 3.7	0.06

CHO, carbohydrate; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid
All values are presented as mean (SE)
* Received daily capsule containing 5 mg folic acid plus 80 mg pyridoxine.
† Received daily capsule containing 5 mg folic acid.
‡ Received daily capsule containing lactose.
§ Obtained from analysis of variance.

Table 3
Characteristics of migraine attacks at study baseline and 3 mo after supplementation in the three intervention groups

Variables	Folic acid + pyridoxine*			Folic acid†			Placebo‡			*P* value§
	Baseline	3 mo	Change	Baseline	3 mo	Change	Baseline	3 mo	Change	
Severity (VAS)‖	8.93 (0.02)	6.22 (0.08)	−2.71 (0.08)¶,#,**	9 (0.01)	6.8 (0.05)	−2.2 (0.05)*	8.98 (0.02)	6.78 (0.05)	−2.19 (0.05)*	<0.001
Frequency (per mo)	12.16 (0.11)	8.8 (0.13)	−3.35 (0.09)¶,#,**	12.41 (0.16)	9.56 (0.15)	−2.85 (0.08)*	12.34 (0.2)	9.6 (0.21)	−2.73 (0.05)*	<0.001
Duration (h)	22.79 (0.15)	15.54 (0.14)	−7.25 (0.17)¶,#,**	22.77 (0.15)	16.38 (0.12)	−6.39 (0.13)*	22.69 (0.15)	16.19 (0.12)	−6.5 (0.07)*	<0.001
HDR††	193.41 (0.19)	119.26 (0.23)	−74.15 (0.2)¶,#,**	193.66 (0.25)	120.9 (0.22)	−72.76 (0.17)*	193.52 (0.25)	120.79 (0.22)	−72.73 (0.1)*	<0.001

HDR, headache diary result; VAS, visual analog scale
All values are presented as mean (SE)
* Received daily capsule containing 5 mg folic acid plus 80 mg pyridoxine.
† Received daily capsule containing 5 mg folic acid.
‡ Received daily capsule containing lactose.
§ Obtained from analysis of variance.
‖ Measured by VAS that ranked the headache severity from 1 to 10.
¶ Significant 3 mo in comparison to baseline: obtained from paired sample *t* test.
# Significant in comparison to placebo group: obtained from independent sample *t* test.
** Significant in comparison to folic acid group: obtained from independent sample *t* test.
†† Determined by formula: frequency × duration.

Intervention

An earlier study revealed that intake of 2 mg folic acid and 25 mg pyridoxine over a 6-mo period decreased the severity and frequency of migraine attacks [13]. The present study aimed to examine a higher dosage of folic acid and pyridoxine on characteristics of migraine attacks. Therefore, patients in the folic acid plus pyridoxine group received daily capsules containing 5 mg folic acid and 80 mg pyridoxine; those in folic acid–only group received daily capsules containing 5 mg folic acid; and those in placebo group received daily capsules containing lactose for 3 mo. In addition to supplements, all patients received routine treatment for migraine headache, which included taking medications (valproate, topiramate, propranolol) that they had previously been taking. No changes were made in medications during the intervention compared with before the intervention. We used a dosage of pyridoxine lower than the upper limit dosage determined for this vitamin (100 mg/d) [18] to reduce unexpected side effects. In terms of folic acid, although the upper limit is 1 mg/d, consumption of 5 mg/d showed no side effects in patients with migraine [18,19]. Therefore, we expected no side effects with this dosage. In terms of placebo production, placebo capsules were similar in color, appearance, and taste to folic acid plus pyridoxine and folic acid capsules, which were produced in the School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran. Packages containing capsules were given to participants by a co-researcher who was not aware of patients' assignment. Patient compliance was measured by counting the remaining capsules at the end of the study using the following formula:

number of used capsules/all given capsules × 100.

Patients were asked not to change their physical activity or dietary intake and not to take any supplement other than those prescribed throughout the study. To assess dietary intake during the study, we obtained 3-d dietary records. Patients were instructed to record as accurately as possible everything they consumed during the day, including the supplement and between-meal and late-evening snacks. The dietary records were based on estimated values in household measurements. We used Modified NUTRITIONIST 4 (version 1) to obtain nutrient intake on the basis of these 3-d dietary records. The reliability and validity of 3-d food record for Iranian adults was confirmed previously [20,21].

Statistical analysis

Quantitative variables were shown as mean and SD, and qualitative variables were represented as number of frequency and their percent. Kolmogrov–Smirnov test was used to examine normal distribution of variables. Log transformation was done for non-normally distributed variables. To examine differences in qualitative variables among the three groups, we used χ^2 test. Analysis of variance was applied to detect differences in general characteristics, dietary intakes, and between-group changes among the three groups. Additionally, we used paired sample *t* test for within-group comparison. Multivariate analysis of covariance was used to examine the effects of vitamin supplementation on characteristics of migraine attacks. In this analysis, all of the baseline values and confounding variables were adjusted to avoid potential bias and to detect independent results. All statistical analyses were done by means of SPSS software version 18 (SPSS, Inc. Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

Follow-up

Of 102 patients participating in the study, three in the folic acid group and four in the placebo group were excluded due to a change medications (n = 2), gastrointestinal disorders (n = 2), and personal reasons (n = 3). Therefore, 95 patients (folic acid

Table 4
Adjusted changes in characteristics of migraine attacks in 3 intervention groups

Variables	Folic acid + pyridoxine group (n = 34)*	Folic acid group (n = 31)†	Placebo group (n = 30)‡	*P* value§
Severity (VAS)‖	−2.83 (0.13)¶,#	−2.19 (0.11)	−2.16 (0.11)	0.003
Frequency (per mo)	−3.36 (0.15)¶	−2.83 (0.13)	−2.72 (0.13)	0.02
Duration (hour)	−7.14 (0.24)	−6.51 (0.20)	−6.53 (0.21)	0.22
HDR**	−74.39 (0.32)¶,#	−72.65 (0.28)	−72.47 (0.28)	0.001

HDR, headache diary result; VAS, visual analog scale
All values are presented as mean (SE) adjusted for baseline values and dietary intakes throughout the intervention
* Received daily capsule containing 5 mg/d folic acid plus 80 mg/d pyridoxine.
† Received daily capsule containing 5 mg/d folic acid.
‡ Received daily capsule containing lactose.
§ Obtained from multivariate analysis of covariance.
‖ Measured by VAS that ranked the headache severity from 1 to 10.
¶ Significant in comparison to placebo group.
# Significant in comparison to folic acid group.
** Determined by formula: frequency × duration.

plus pyridoxine group [n = 34], folic acid group [n = 31], placebo group [n = 30]) completed the study and were considered for final analysis (Fig. 1). Migraine patients in the three intervention groups consumed all of the prescribed capsules throughout the trial, thus the rate of compliance in this trial was 100%.

Primary outcome

Baseline characteristics of migraine patients in the three groups are presented in Table 1. Mean (± SD) age and BMI of participants was 35.21 ± 10.4 y and 25.93 ± 4.75 kg/m^2, respectively. At the beginning of study, there were no significant differences in sex distribution, age, BMI, homocysteine level, characteristics of migraine attacks (headache severity, attacks frequency, duration, and HDR), and family history of migraine among the intervention groups.

On the basis of 3-d dietary records obtained throughout the intervention, no significant differences were found in dietary intakes of fruits, nuts, citrus, riboflavin, pyridoxine, folate, cobalamin, magnesium, sodium, saturated fatty acids, poly-unsaturated fatty acids, linoleic acid, linolenic acid, or ω-6/3 among the three groups. However, intake of dairy, tomato, chocolate, energy, protein, carbohydrate, fat, and mono-unsaturated fatty acids did differ. These differences in intake of energy, foods, and nutrients were adjusted in the final analysis (Table 2).

Secondary outcome

At the end of this trial, all characteristics of migraine attacks in the three groups decreased significantly compared with baseline. Compared with the placebo group, folic acid plus pyridoxine intake resulted in a significant decrease in headache severity (−2.71 ± 0.08 versus −2.19 ± 0.05; $P < 0.001$), attack frequency (−3.35 ± 0.09 versus −2.73 ± 0.05; $P < 0.001$), duration (−7.25 ± 0.17 versus −6.5 ± 0.07; $P < 0.001$), and HDR (−74.15 ± 0.2 versus −72.73 ± 0.1; $P < 0.001$). Additionally, the reduction in these characteristics of migraine attacks in the folic acid plus pyridoxine group was significant compared with the folic acid–only group. However, folic acid intake without pyridoxine did not lead to a significant decrease in migraine symptoms compared with the placebo group (Table 3). When the analyses were adjusted for baseline values and dietary intake during the trial, no significant changes were observed for headache severity, attack frequency, and HDR; however, the reduction of attack frequency in the combination group was not significant compared with folic acid–only group. Additionally, the beneficial effects of the combination supplementation on duration of migraine attacks became nonsignificant after adjusting for confounders (Table 4).

Adverse effects

In this study, two patients reported heartburn as a side effect of the vitamin intake. In these cases, consumption of the vitamin was stopped and they were excluded from the trial.

Discussion

The present study demonstrated that folic acid intake combined with pyridoxine decreases headache severity, attack frequency, and HDR in patients with MA; however, folic acid intake alone had no effect on these characteristics. To the best of our knowledge, this was the first study to examine the effects of folic acid alone and in combination with pyridoxine on characteristics of migraine attacks. It also may be the first to examine the effects of these vitamins on duration of migraine attacks and HDR. Previous studies [12,13] mostly focused on combination effects of these vitamins on MA symptoms, and data on the effects of single vitamin B supplementations on these symptoms are scarce. A recent randomized, double-blind, placebo-controlled clinical trial showed that 6-mo supplementation with pyridoxine (25 mg), folic acid (2 mg), and cobalamin (400 μg) in 52 migraine patients caused a reduction in severity and frequency of migraine attacks as well as migraine disabilities [12]. In another similar clinical trial, consumption of pyridoxine, folic acid, and cobalamin over a 6-mo period decreased headache severity and migraine disabilities, although frequency of migraine attacks did not decrease significantly [13]. In contrast with the present findings, Di Rosa et al. reported that supplementation with folic acid in children with migraine and hyperhomocysteinemia led to a decrease in the frequency of migraine attacks as well as a reduction in homocysteine levels [19]. In the present study, mean homocysteine levels at baseline were normal; therefore, we did not measure this level at the end of the trial. Based on our findings, intake of folic acid plus pyridoxine reduced attack duration and HDR. However, the effect on duration of the migraine attack became nonsignificant after controlling for potential confounders. We are aware of no other studies that examined the effects of folic acid plus pyridoxine intake on these characteristics of migraine attacks.

The exact mechanism explaining the beneficial effects of folic acid plus pyridoxine intake on MA symptoms is not known. Published studies reported that folic acid and pyridoxine deficiencies may be present in migraine patients, and therefore intake of these vitamins reduces the migraine symptoms by eliminating this deficiency [12,13]. Additionally, it has been shown that migraine, especially MA, is associated with point mutation in the *MTHFR* gene. Without considering homocysteine level, this mutation is associated with high severity and frequency of migraine attack [12]. It has been shown that intake of folic acid and pyridoxine in high doses reduces the effects of this mutation [22]. However, consumption of these vitamins in a high dosage can decrease migraine symptoms by reducing homocysteine levels [12,13]. Additionally, experimental studies have shown that B vitamins in their separate forms (B_1, B_6, and B_{12}) have antinociceptive or analgesic effects in the model of pain induced by acetic acid in mice [23]. Further clinical trials are required to examine this hypothesis on migraine patients.

Some limitations of our study need to be taken account. As a result of limited funding, we did not measure the plasma pyridoxine and folic acid levels at baseline and at the end of the trial. Moreover, we could not examine the effects of folic acid plus pyridoxine supplementation on other MA symptoms such as nausea, vomiting, and sensitivity to light and sound. Also due to small sample size, we were unable to examine whether the favorable effects of folic acid plus pyridoxine supplementation is different between men and women. Furthermore, the appropriate dosage of folic acid plus pyridoxine in patients with MA cannot be inferred from this study and further studies are required.

Conclusions

Folic acid intake in combination with pyridoxine decreased the characteristics of migraine attacks including headache severity, attack frequency, and HDR. Consumption of folic acid

alone had no effects on these characteristics. Further studies are needed to shed further light on these findings.

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