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Effects of probiotic and vitamin D co-supplementation on clinical symptoms, mental health, and inflammation in adult patients with migraine headache: a randomized, triple-blinded, placebo-controlled trial

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Abstract

Background Migraine headache is a major public health problem. Routine medications for migraine treatment are not useful in treating all patients and may have some side effects. The present study aimed to investigate the effect of vitamin D and probiotic co-supplementation on clinical characteristics of migraine, daily functioning, mental health outcomes, and serum levels of high-sensitivity C-reactive protein (hs-CRP).

Methods In this randomized, triple-blinded, placebo-controlled trial, patients aged 18 to 55 years diagnosed with migraine based on the International Classification of Headache Disorders-3 (ICHD-3) were randomized to either vitamin D (50,000 IU every 2 weeks) plus probiotic (4.5×10^{11} CFU per day) or placebo for 12 weeks. The Headache Impact Test (HIT-6) and Depression, Anxiety, and Stress Scale (DASS) questionnaires were administered to patients at baseline and after 12 weeks. In addition, the frequency, duration, and severity of migraine headaches per month were assessed using a self-administered 30-day headache diary at baseline and the end of the intervention. Anthropometric indices, blood pressure, and serum levels of 25-hydroxy vitamin D and hs-CRP were also examined at first and the end of the study.

Results Seventy-two migraine patients with a mean age of 37.46 ± 8.32 years were included in this trial. Probiotic and vitamin D co-supplementation compared to placebo resulted in a significant increase in serum levels of vitamin D ($+12.86\pm1.64$ vs. $+1.12\pm0.80$ ng/mL, $P<0.001$). The between-group analysis in the adjusted model showed a significantly greater reduction in migraine headache frequency (-3.17 ± 0.84 vs. -1.25 ± 0.34; $P=0.031$) and severity (-1.55 ± 0.35 vs. $+0.67\pm0.29$; $P=0.017$) in the probiotic and vitamin D group than the placebo group. No significant difference was found between the two arms of the intervention regarding the change in headache duration, hs-CRP, scores of DASS, and HIT-6 questionnaires ($P>0.05$).

Conclusions This trial showed that probiotic and vitamin D co-supplementation for 12 weeks has beneficial effects on migraine headache characteristics. Further research is needed to confirm this finding.

Keywords Migraine headache, Probiotic, Vitamin D, Inflammation, Mental health, Randomized clinical trial

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Background

Migraine is a primary headache affecting more than 1 billion individuals all around the globe [1]. Clinically, migraine is diagnosed by the third edition of the International Classification of Headache Disorders (ICHD-3) [2]. Migraine is defined according to the criteria as recurrent headaches with two of four headache characteristics (unilateral, pulsating, moderate to severe, and aggravating by routine physical activity) plus one of the correlated symptoms (nausea and/or vomiting, phonophobia, and photophobia) during attacks. Patients with less than 15 headache days per month are considered to suffer from episodic migraine (EM). However, patients with 15 or more days of headache (per month), comprising at least 8 days of headache with migraine features, for more than 3 consecutive months have chronic migraine (CM). Nearly, one-third of migraine patients represent attacks with aura which are known as reversible focal neurological symptoms that develop over 5 to 60 min and usually occur before the headache phase [2].

According to the Global Burden of Disease (GBD) 2019, migraine is one of the leading reasons for years living with disability (YLDs) [3]. Furthermore, robust evidence from previous research has indicated that the prevalence of psychiatric comorbidities, such as depression, anxiety, and sleep disorders, is higher in migraine patients compared to the general population, which, unfavorably affects the quality of life (QoL) and clinical outcomes in migraineurs [4–6]. Although several medications are available to alleviate pain and clinical symptoms of migraine, acute treatment with most of them might not lead to favorable clinical outcomes [7, 8]. Suboptimal treatment can lead to medication overuse, which, in turn, increases the chronicity of the disease and the risk of depression and anxiety [9–11]. As a result, migraine imposes a substantial burden not only on individuals and their families but also on societies because of direct medical costs of the disease and indirect costs related to unemployment and lost work time [12–14]. Thus, exploring more effective treatment options is crucial for the efficient management of migraine.

The pathophysiology of migraine is complex and its underlying mechanisms are not fully understood. However, recent data suggest that the gut-brain axis may play a role in the pathophysiology of the headache [15–18]. Migraine may be linked to gastrointestinal (GI) comorbidities such as diarrhea, constipation, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), Helicobacter pylori infection, and disorders of gut-brain interaction (DGBI) such as functional dyspepsia and cyclic vomiting syndrome (CVS) [19–23]. Furthermore, increased levels of pro-inflammatory cytokines (such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6, and high sensitivity C-reactive protein (hs-CRP)) resulting from GI microbiota dysbiosis and increased gut permeability have been observed during migraine attacks. Additionally, some neuropeptides such as serotonin are involved in the migraine pathogenesis through the gut-brain axis [15]. Therefore, it seems that therapeutic strategies affecting the intestinal microbiota can be useful in the treatment of migraine.

Probiotics could probably relieve migraine headaches through improving the function of gut-brain axis. Few previous studies have investigated the effect of probiotic supplementation on migraine attacks; however, there was no consensus on their results [24, 25]. Recent evidence has also implied the influence of vitamin D on gut microbiota [26]. Vitamin D supplementation has also shown beneficial effects on the frequency and severity of migraine attacks [27–29]. It seems that one of the mechanisms through which vitamin D improves migraine attacks might be through its effect on the brain-intestinal axis [30]. We hypothesized that probiotic and vitamin D supplementation might be synergistically effective on the brain-gut axis and migraine symptoms. Thus, the objective of the present study was to evaluate the effect of probiotic and vitamin D co-supplementation on the frequency, duration, and severity of migraine attacks, daily functioning, mental health outcomes, and serum levels of hs-CRP in adult patients with migraine.

Methods

Study design and patients

The present study was a parallel randomized, triple-blinded, placebo-controlled trial conducted in a central neurology clinic in Isfahan city, Iran. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences (no.3401664) and a written informed consent form was signed by each patient before study initiation. The study protocol was also registered at the Iranian Registry of Clinical Trials (no. IRCT20121216011763N59).

Sample size was calculated using the mean difference and standard deviation for migraine headache frequency, as the key variable, based on a previous study considering a power of 90% and type I error of 0.05 [25]. The minimum sample size was estimated to be 35 patients in each group. Patients were considered eligible for inclusion in the trial if they were between 18 and 55 years old, had a history of migraine with or without aura based on the ICHD-3, and experienced more than 2 migraine attacks per month during the 3 preceding months. Non-inclusion criteria were pregnancy or breastfeeding; non-migraine headaches; drug overuse headache; clinical diagnosis of endocrine, cardiovascular, neurological, renal, hepatic, and gastrointestinal diseases (like ulcerative colitis and

Crohn's disease); the use of vitamin D supplements, probiotic supplements, probiotic-fortified foods, and antibiotics up to 3 months before the initiation of the study. Patients with any change in their treatment approach, i.e., modifications in the type or dose of prophylactic medications, adherence rate lower than 80%, and those who were not willing to continue the intervention were excluded from the trial.

Randomization and intervention

Patients were randomly assigned to the probiotic plus vitamin D supplementation group or placebo group, according to a scheme generated by a web-based system using a permuted block with a size of four (https://www.sealedenvelope.com/). All patients, investigators, and analyzers were blinded to the randomization list and administered drugs until the end of statistical analysis. An unblinded investigator who was not involved in study assessments labeled probiotic and vitamin D supplements. Patients in the intervention group received a probiotic capsule (4.5×10^{11} CFU) (Farabiotic Pharmaceutical Company, Tehran, Iran) per day and a pearl of vitamin D (50,000 IU) (Zahravi Pharmaceutical Company, Tabriz, Iran) every 2 weeks for 12 weeks. However, patients in the control group received a placebo capsule for probiotic (containing starch and maltodextrin) every day and a placebo pearl for vitamin D (containing corn oil) every 2 weeks for 12 weeks. Supplement and placebo capsules (or pearls) were identical in packaging, size, shape, and color. The probiotic supplement contained eight different strains of *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium infantis*, *Bifidobacterium longum, Bifidobacterium breve,* and *Streptococcus thermophilus.* To increase the compliance of patients, dates of vitamin D use were provided to each patient and he/she received weekly phone reminders.

Migraine headache assessment

Patients were provided with a 1-month headache diary and asked to fill in the information regarding headache severity (based on the visual analog scale (VAS)), duration (hour), and frequency of attacks per month. Patients were instructed to fulfill the diaries 1 month before the initiation of the intervention and in the last month of the intervention (weeks 8 to 12).

The short-from Headache Impact Test-6 (HIT-6) was used to evaluate the ability of normal functioning in daily life at baseline and at the end of the intervention [31]. This validated tool encompasses 6 questions in the domains of social role functioning, pain, emotional distress, well-being, cognitive functioning, and vitality. To score responses following values were used: never = 6, rarely = 8, sometimes = 10, very often = 11, and always = 13. The final HIT-6 scores of 36–49, 50–55, 56–59, and ≥ 60 indicated that headache has no, moderate, substantial, and severe impact, respectively.

Biochemical variables

Blood samples were collected after 12 h of fasting at baseline and the end of the intervention. After 10 min of centrifuge at 3500 rpm, serum was separated and kept at − 80 °C for future assessments. Serum levels of 25-hydroxy vitamin D were measured using an ELISA commercial kit (DiaZist company, Tehran, Iran). High-sensitivity C-reactive protein (hs-CRP) was examined by a commercial kit according to the turbidimetric method. Biochemical variables were measured at baseline and the end of the intervention.

Blood pressure and anthropometric indices

Blood pressure (BP) was measured in a sitting position after a 10-min resting using a digital sphygmomanometer (Rossmax Swiss GmbH, Heerbrugg, Switzerland) in the fasting condition. BP measurement was repeated two times for each patient 5 min apart and the mean of two measurements was used for data analysis. Mean arterial pressure (MAP) was computed as 1/3 systolic blood pressure (SBP) plus 2/3 diastolic blood pressure (DBP). Anthropometric variables were measured by a trained dietitian, while subjects were standing in light clothing, with bare feet. Weight (kg) was measured by a digital scale (Omron, HN-286, Kyoto, Japan) to the nearest 0.1 kg, and height (cm) was measured by a wall-mounted tape measure to the nearest 0.1 cm. Body mass index (BMI) (kg/m^2) was estimated by dividing weight by the height squared. The measurements of BP and anthropometric variables were performed at baseline and the end of the intervention.

Other variables

A standard checklist was used in the first visit to gather information regarding patients' age, sex, marital status, number of households, family history of migraine, medication use, smoking, education, and menopausal status. Patients were asked to complete 6 dietary records (1 record every 2 weeks) during the intervention to evaluate their dietary intakes. The reported portion sizes of consumed foods were converted to grams per day using household measures. The daily intake of energy and nutrients was computed using the Nutritionist IV software. Patients also recorded their physical activity in two non-consecutive days. Physical activity data collected by these records were converted to Metabolic Equivalent Task hours per day (MET.hr/d).

The 21-item Depression, Anxiety, and Stress Scale (DASS) questionnaire was used to estimate the psychological characteristics of patients both at baseline and after 12 weeks of the intervention. This questionnaire has shown acceptable psychometric properties of validity, internal consistency, and test–retest reliability among the Iranian adult population [32]. The tool evaluates depression, anxiety, and distress during the last week. Each subscale contained 7 questions rating on a 4-point Likert scale ranging from 0 (not at all) to 3 (very much) with a total score varied from 0 to 21 for each subscale. Higher scores for each subscale represented higher symptoms of depression, anxiety, and distress.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normality of continuous variables. Data were reported as mean (SD or SE) for quantitative variables and frequency (percentage) for qualitative variables. Baseline characteristics were compared between two groups using independent samples *t*-test and Pearson's chi-square test for continuous variables and qualitative variables, respectively. Furthermore, a paired sample *t*-test was used to evaluate within-group changes of variables during 12 weeks of intervention. To assess differences between two intervention groups, independent samples *t*-test and analysis of covariance (ANCOVA) were applied. Taking tricyclic antidepressants (TCAs), taking triptans, and baseline values of MAP were considered as covariates. Both per-protocol and intention-to-treat (ITT) analyses were performed. The last-observation-carried-forward method was used to treat missing values in ITT analyses. SPSS software version 20 (IBM, Chicago, IL) was used to perform statistical analyses and a P-value < 0.05 (two-tailed) was considered statistically significant.

Results

In total, 72 patients with a mean age of 37.46 ± 8.32 (SD) years and mean BMI of 25.04 ± 3.26 (SD) kg/m^2 were included in the present study. At the end of the trial, 68 patients completed the study protocol and 4 individuals were excluded due to poor adherence ($n=1$) and declining to continue the intervention ($n=3$). Individuals who completed the trial ($n=68$) were included in the pre-protocol analysis. In addition, ITT analysis was performed for all 72 patients by replacing the missing data with the last observed value. The follow-up process is depicted in Fig. 1.

Baseline characteristics of participants are summarized in Table 1. The mean SBP (109.08 vs.

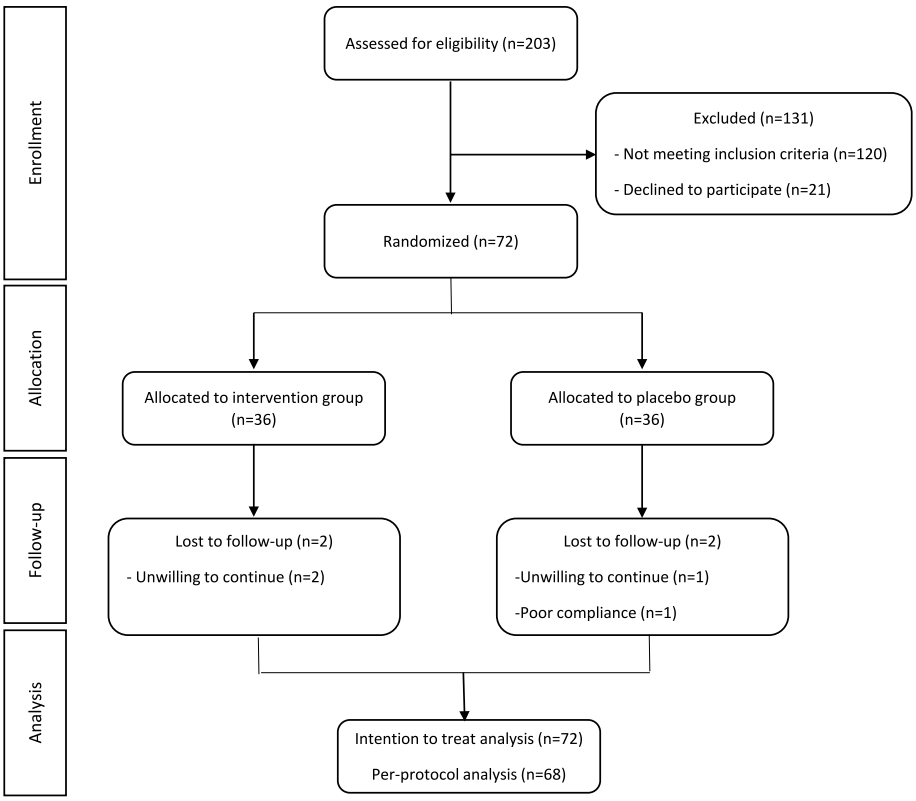

Fig. 1 Patients' flow diagram

Table 1 Baseline demographic and clinical characteristics of study participants in two intervention groups ($n=72$)

Variables		Probiotic and vitamin D ($n=36$)	Placebo ($n=36$)	*P*-value [1]
Age (year)		37.44±8.84	37.47±7.90	0.989
Sex				
	Male	3 (8.3)	4 (11.1)	0.999
	Female	33 (91.7)	32 (88.9)	
Education				
	Diploma or lower	17 (47.2)	20 (55.6)	0.638
	University education	19 (52.8)	16 (44.4)	
Marital status				
	Single	7 (19.4)	9 (25.0)	0.731
	Married	27 (75.0)	24 (66.7)	
	Divorced or widow	2 (5.6)	3 (8.3)	
Family size				
	< 4	15 (41.7)	13 (36.1)	0.809
	≥ 4	21 (58.3)	23 (63.9)	
Body weight (kg)		67.81±11.04	67.32±10.98	0.851
BMI (kg/m^2)		24.97±3.03	24.89±3.52	0.914
SBP (mmHg)		109.08±13.29	115.08±9.31	0.030
DBP (mmHg)		75.92±6.74	79.08±6.53	0.047
MAP		86.97±8.19	91.08±6.54	0.021
Smoking		2 (5.6)	0 (0.0)	0.309
Postmenopausal		2 (5.6)	4 (11.1)	0.674
History of migraine in first degree family		8 (22.2)	9 (25.0)	0.999
Migraine with aura		14 (38.9)	21 (58.3)	0.157
Prophylactic medications for migraine				
	TCAs	15 (41.7)	25 (69.4)	0.032
	Beta-blockers	13 (36.1)	12 (33.3)	0.999
	Triptans	15 (41.7)	6 (16.7)	0.037
	SNRIs	4 (11.1)	3 (8.3)	0.999
	Benzodiazepines	4 (11.1)	3 (8.3)	0.999
	Gabapentin	3 (8.3)	4 (11.1)	0.999
	Topiramate	2 (5.6)	1 (2.8)	0.999
	Sodium valproate	2 (5.6)	1 (2.8)	0.999

Abbreviations: BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *TCAs* tricyclic antidepressants, *SNRIs* serotonin and norepinephrine reuptake inhibitors

[1] Resulted from independent-samples *t*-test for quantitative variables and chi-square test (Fisher's exact test) for categorical variables

Quantitative variables: mean ± SD. Qualitative variables: frequency (percentage)

115.08 mmHg; $P=0.030$), DBP (75.92 vs. 79.08 mmHg; $P=0.047$), and MAP (86.97 vs. 91.08 mmHg; $P=0.021$) values of individuals were significantly lower in the probiotic and vitamin D group than that in the placebo group. Furthermore, there was a significant difference between the two groups regarding taking triptans (probiotic and vitamin D vs. placebo: 41.7 vs. 16.7%; $P=0.037$) and TCAs (probiotic and vitamin D vs. placebo: 69.4 vs. 41.7%; $P=0.032$). No statistically significant differences were observed between the probiotic and vitamin D and placebo groups regarding other baseline demographic and clinical characteristics ($P>0.05$).

The dietary intakes of participants through the intervention are presented in Table 2. No significant difference has been observed between the two groups regarding dietary intakes of energy, macronutrients, and micronutrients such as cholesterol, magnesium, calcium, vitamin C, and vitamin E ($P>0.05$). There was also no significant difference between the probiotic and vitamin D and the placebo groups regarding the level of physical activity (35.22 ± 7.54 vs. 33.34 ± 10.16 MET.hr/d; $P=0.413$).

Table 2 Daily energy and nutrient intakes of patients throughout the study ($n=72$)[a]

Variables	Probiotic and vitamin D ($n=36$)	Placebo ($n=36$)	*P*-value [1]
Energy (kcal)	1966.00 ± 93.10	1869.71 ± 107.89	0.500
Carbohydrate (g)	235.75 ± 14.37	235.83 ± 14.84	0.997
Protein (g)	65.91 ± 3.92	68.92 ± 5.52	0.654
Fat (g)	87.39 ± 4.34	79.72 ± 4.60	0.230
Dietary fiber (g)	13.60 ± 1.08	14.19 ± 1.17	0.713
Cholesterol (mg)	257.12 ± 20.68	277.18 ± 31.78	0.592
Magnesium (mg)	164.59 ± 11.02	170.88 ± 13.43	0.716
Calcium (mg)	563.99 ± 40.02	505.06 ± 31.80	0.260
Vitamin C (mg)	78.97 ± 8.72	75.06 ± 9.10	0.428
Vitamin E (mg)	34.77 ± 2.80	29.42 ± 1.87	0.118

[1] Resulted from independent-samples *t*-test

[a] All values are presented as mean ± SE

Furthermore, the changes in weight (probiotic and vitamin D vs. placebo: + 1.35 ± 0.26 vs. + 1.00 ± 0.50 kg; *P* between group = 0.542) and BMI (probiotic and vitamin D vs. placebo: + 0.50 ± 0.10 vs. + 0.35 ± 0.19 kg/m^2; $P=0.488$) between the two arms of the intervention were not statistically significant after 12 weeks.

As shown in Fig. 2, probiotic and vitamin D co-supplementation compared to placebo resulted in a significant increase in serum levels of vitamin D (+ 12.86 ± 1.64 vs. + 1.12 ± 0.80 ng/mL, $P<0.001$), exhibiting high adherence of participants to the intervention. Table 3 presents the effect of probiotic and vitamin D co-supplementation on migraine headache frequency, duration, and severity as well as the HIT-6 score, based on ITT analyses. Within-group analyses revealed that migraine headache frequency, duration, and severity as well as HIT-6 score were significantly decreased in both groups compared to the baseline values ($P<0.05$). The results of between-group analyses showed a significant reduction in migraine headache frequency in the probiotic and vitamin D group versus the control group (− 3.17 ± 0.84 vs. − 1.25 ± 0.34; $P=0.039$). In the case of migraine headache duration (− 7.70 ± 2.38 vs. − 3.01 ± 1.24; $P=0.086$) and severity (− 1.55 ± 0.35 vs. − 0.67 ± 0.29; $P=0.057$), changes between the two groups were marginally significant. However, no significant difference was found in HIT-6 score changes between the two groups (− 5.94 ± 1.29 vs. − 4.94 ± 1.13; $P=0.562$). Analysis of covariance (with adjustments for baseline MAP, taking triptans, and taking TCAs) revealed a significant difference between the two groups in terms of reductions in migraine headache frequency ($P=0.031$) and severity ($P=0.017$). However, there was no significant difference in migraine headache duration and HIT-6 score changes between the two groups ($P=0.171$). Similar findings were found based on per-protocol analyses on 68 patients (Additional File 1: Table S1).

Regarding the scores of depression, anxiety, and distress, there was no significant difference between two groups of intervention at baseline (data not shown). Table 4 describes the effect of probiotic and vitamin D co-supplementation on mental health indices of depression, anxiety, and distress (based on ITT analyses). According to within-group analyses, distress score was significantly decreased in the probiotic and vitamin (23.50 ± 1.76 vs. 27.44 ± 1.56; $P=0.014$) and placebo (24.89 ± 1.85 vs. 28.78 ± 1.56; $P=0.002$) groups compared to the baseline values. A marginally significant reduction was also observed in anxiety scores in both probiotic and vitamin D (14.17 ± 1.67 vs. 16.94 ± 1.68; $P=0.064$) and placebo (16.61 ± 1.77 vs. 18.55 ± 1.85; $P=0.074$) groups. Additionally, depression score

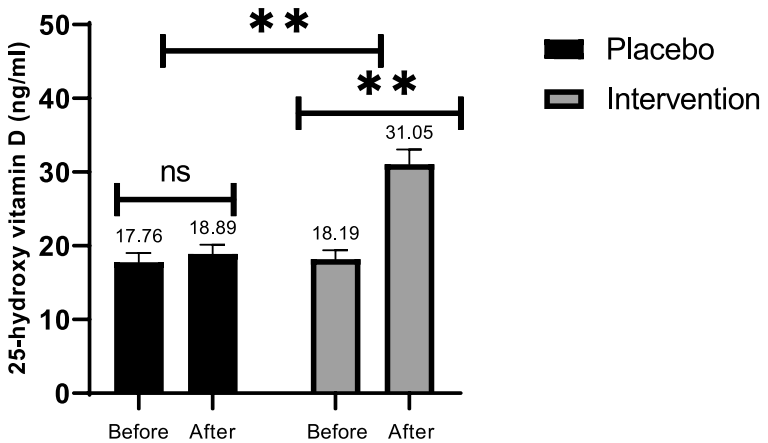

Fig. 2 Serum levels of 25-hydroxy vitamin D (ng/mL) at baseline and after 12 weeks of intervention in migraine patients. Values and mean ± SE. * indicates $P<0.05$, ** indicates $P<0.001$, and "ns" indicates non-significant

Table 3 Effects of probiotic and vitamin D co-supplementation on migraine symptoms and HIT-6 score (based on intention to treat analyses, $n=72$)[d]

Variables	Probiotic and vitamin D ($n=36$)	Placebo ($n=36$)	Mean difference	*P*-value[b]	*P*-value[c]
Migraine attacks (frequency/month)					
Baseline	7.76±0.81	5.97±0.67			
12th week	4.50±0.56	4.72±0.67	−1.92±0.90	0.039	0.031
Mean change	−3.17±0.84	−1.25±0.34			
P-value[a]	0.001	0.001			
Migraine duration (hours/month)					
Baseline	23.26±3.55	23.10±3.69			
12th week	16.56±3.15	20.10±3.25	−4.69±2.69	0.086	0.171
Mean change	−7.70±2.38	−3.01±1.24			
P-value[a]	0.003	0.020			
Migraine severity (based on VAS)					
Baseline	8.31±0.28	8.36±0.22			
12th week	6.75±0.39	7.69±0.32	−0.89±0.46	0.057	0.017
Mean change	−1.55±0.35	−0.67±0.29			
P-value[a]	<0.001	0.030			
HIT-6 score					
Baseline	65.31±0.97	65.47±0.75			
12th week	59.36±1.50	60.53±1.08	−1.00±1.72	0.562	0.726
Mean change	−5.94±1.29	−4.94±1.13			
P-value[a]	<0.001	<0.001			

Abbreviations: HIT-6 Headache Impact Test-6, *VAS* visual analog scale

[a] Resulted from paired *t*-test for comparison of within-group changes

[b] Resulted from independent-samples t-test for comparison of between-group differences

[c] Resulted from ANCOVA; adjusted for taking tricyclic antidepressants, triptans, and baseline mean arterial pressure

[d] All values are presented as mean ± SE

was significantly reduced in the probiotic and vitamin D group (17.44 ± 1.86 vs. 21.28 ± 1.75; $P=0.008$); however, no significant change was observed in depression score in the placebo group (19.44 ± 2.21 vs. 21.17 ± 1.83; $P=0.125$). The results of between-group analyses and analysis of covariance exhibited no statistically significant differences between the two groups in terms of changes in mental health indices ($P>0.05$). The same findings were derived from per-protocol analyses (Additional File 1: Table S2).

Figure 3 shows the effect of probiotic and vitamin D co-supplementation on serum levels of hs-CRP in migraine patients. Based on ITT analysis (Fig. 3A), a non-significant decrease was observed in serum levels of hs-CRP in the probiotic and vitamin D (1.53 ± 0.23 vs. 1.85 ± 0.22; $P=0.261$) and placebo (1.51 ± 0.20 vs. 1.84 ± 0.26; $P=0.382$) groups compared to the baseline values. No significant difference was observed in hs-CRP reduction between the two groups according to between-group analyses ($P=0.959$) and analysis of covariance ($P=0.784$). The same results were derived from per-protocol analysis (Fig. 3B).

No side effect related to probiotic and vitamin D co-supplementation was reported neither in the probiotic and vitamin D group nor in the placebo group.

Discussion

Our findings showed that probiotic and vitamin D co-supplementation for 12 weeks could result in a significant reduction in migraine headache frequency and slight decreases in migraine headache duration and severity. After taking covariates into account, only migraine headache frequency and severity were substantially decreased by this intervention. Probiotic and vitamin D co-supplementation had no effects on daily functioning, mental health outcomes, and serum levels of hs-CRP in adult patients with migraine.

The present study, to the best of our knowledge, is the first randomized clinical trial that investigated the effect of probiotic and vitamin D co-supplementation on clinical and psychological features of migraine headaches. The findings of the present study are consistent with a previous randomized clinical trial by Martami et al., which investigated the effect of a multispecies probiotic

Table 4 Effects of probiotic and vitamin D co-supplementation on depression, anxiety, and depression (based on intention to treat analyses, $n=72$)[d]

Variables	Probiotic and vitamin D ($n=36$)	Placebo ($n=36$)	Mean difference	*P*-value[b]	*P*-value[d]
Depression					
Baseline	21.28±1.75	21.17±1.83			
12th week	17.44±1.86	19.44±2.21	−2.11±1.74	0.229	0.303
Mean change	−3.83±1.35	−1.82±1.16			
P-value[a]	0.008	0.125			
Anxiety					
Baseline	16.94±1.68	18.55±1.85			
12th week	14.17±1.67	16.61±1.77	−0.83±1.79	0.644	0.857
Mean change	−2.78±1.45	−1.94±1.05			
P-value[a]	0.064	0.074			
Distress					
Baseline	27.44±1.56	28.78±1.56			
12th week	23.50±1.76	24.89±1.85	−0.06±1.92	0.977	0.837
Mean change	−3.94±1.53	−3.89±1.16			
P-value[a]	0.014	0.002			

[a] Resulted from paired *t*-test for comparison of within-group changes

[b] Resulted from independent-samples *t*-test for comparison of between-group differences

[c] Resulted from ANCOVA; adjusted for taking tricyclic antidepressants, triptans, and baseline mean arterial pressure

[d] All values are presented as mean SE

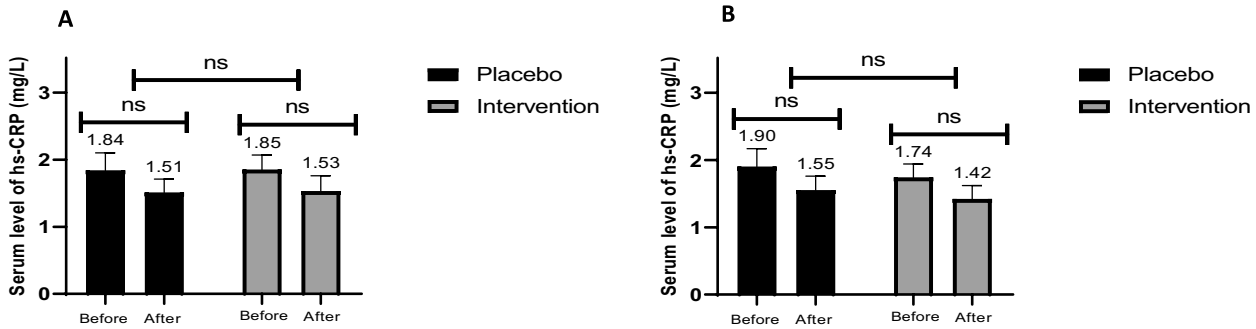

Fig. 3 Effect of probiotic and vitamin D supplementation on serum level of hs-CRP (mg/L) in migraine patients based on intention to treat analysis ($n=72$) (**A**) and per-protocol analysis ($n=68$) (**B**). Values and mean ± SE. * indicates $P<0.05$, ** indicates $P<0.001$, and "ns" indicates non-significant

supplementation (a single daily dose of two capsules; 2.5×10^9 CFU) on clinical characteristics of migraine after 10 and 8 weeks of intervention in patients with episodic and chronic migraine respectively. The mentioned study demonstrated that probiotic supplementation caused a significant reduction in the frequency and severity of migraine headaches compared to the placebo group. However, the results of the study showed a significant reduction in the duration of migraine attacks only in patients with chronic migraine [25]. An open-label study also evaluated the effect of multispecies probiotic supplementation (a single daily dose; 5×10^9 CFU) on 63 patients with migraine headaches for 12 weeks. In contrast to our findings, that study failed to show any significant influence on migraine headache characteristics [24]. The difference in the results of that study is probably due to the difference in the study population, such that the study has been performed on patients with episodic migraine. The dose and bacterial species of the probiotic supplement are also different from the supplement administered in the present study. Additionally, vitamin D may enhance the effectiveness of probiotic supplementation in the present study. However, further research is needed to compare the effects of probiotic and vitamin

D co-supplementation with supplementation with either of these agents alone. Findings of the above-mentioned studies, like our findings, demonstrated no significant difference between the probiotic and placebo groups regarding the changes in inflammatory biomarkers such as TNF-α and CRP [24, 25]. The role of probiotic supplementation on the HIT-6 score has not been investigated previously. HIT-6 measures headache impact on social and role functioning, vitality, cognitive functioning, and psychological distress in patients with migraine [33]. In the present study, probiotic and vitamin D co-supplementation resulted in no significant difference in HIT-6 score compared to the placebo, which can be explained by the possible effect of other factors such as the migraine type and psychological disorders on the score [34–36]. A randomized clinical trial indicated that vitamin D supplementation with a dose of 100 μg/day (or 4000 IU/d) could significantly decrease migraine headache frequency compared to placebo. However, no significant effects on migraine headache severity, number of days with migraine, and HIT-6 score were observed [28]. A marginally significant reduction in the frequency of migraine headaches after 10 weeks of intervention was reported in another randomized clinical trial that investigated the effect of vitamin D supplementation of 50,000 IU/week in migraine patients. However, this study did not report a significant difference in the severity and duration of migraine headache as well as serum levels of CRP [27]. Ghorbani et al., in another randomized clinical trial, showed that daily intake of vitamin D 2000 IU/d resulted in a significant reduction in migraine days per month, migraine attacks duration, and severity than placebo in subjects with episodic migraine [29]. Previous reports regarding the favorable role of vitamin D supplementation on migraine headache are controversial, and are possibly associated with differences in study design, studied patients, baseline vitamin D levels, and supplement dosage. Given the differences in the design of previous studies and the controversial results obtained from them, more randomized clinical trials are needed to discover the efficacy of vitamin D and probiotic supplementation alone or in combination with each other on migraine headache.

Prior investigations have stated that patients with migraine headaches, mainly those with chronic migraine or migraine with aura, are at an increased risk of psychiatric disorders such as anxiety and depression which would increase the burden and decrease treatment response in these patients [37, 38]. No study has previously investigated the effect of probiotic and vitamin D supplementation on mental health outcomes in migraine patients. The results of the present study failed to show any significant effects of probiotics and vitamin D co-supplementation on mental health outcomes. Furthermore, based on the results of recent systematic reviews and meta-analyses, the effect of probiotics [39–41] or vitamin D [42, 43] supplementation on mental health indices in various populations might be doubtful. Thus, further studies should be conducted to investigate the effect of probiotics and vitamin D supplementations alone or in combination with each other on mental health outcomes in different populations, including migraine patients.

The favorable effect of probiotics and vitamin D co-supplementation on some clinical characteristics of migraine headaches can be explained by several potential mechanisms. Previous findings have shown that gut microbiota dysbiosis and increased gut permeability stimulate the release of proinflammatory cytokines which lead to migraine headache initiation by inducing nociceptive responses in the trigeminal pathway [17, 44–46]. Thus, it seems that treatment options affecting gut microbiota such as vitamin D and probiotic supplementation can alleviate migraine headache symptoms by improving gut microbiota dysbiosis and consequent related inflammatory and immune responses. In the present study, we could not find any effect of probiotics and vitamin D co-supplementation on serum levels of hs-CRP as an inflammatory biomarker. Further research is needed to investigate the effect of co-supplementation with vitamin D and probiotics on other inflammatory biomarkers such as IL-6, IL-β, and TNF-α. Additionally, it has been documented that serotonin is a key neurotransmitter in migraine pathophysiology, as serotonin levels decrease between migraine attacks and transiently increase during attacks [47]. The gut microbiota impacts serotonin synthesis and signaling in the GI tract [48, 49]. Therefore, probably one of the mechanisms through which probiotic and vitamin D supplementation can improve migraine headaches is altering gut microbiota and peripheral synthesis of serotonin.

The present study has some strengths and weaknesses. First of all, this is the first randomized clinical trial that investigated the effect of probiotics and vitamin D co-supplementation on adult patients with migraine headaches. Additionally, patients had high adherence to treatment considering regular weekly reminders and serum vitamin D improvement. The present study was performed on both sexes; however, the majority of participants were females, which is due to the higher prevalence of migraine headaches in females than males. However, it limits the generalizability of our findings to males. Several covariates such as blood pressure, weight, menopausal status, and medication use were considered in this trial; nevertheless, other confounders such as exposure to migraine triggers, stressful life events, sleep quality, and sleep duration which may affect our

results remained to be assessed. Furthermore, evaluations of physical activity and dietary intakes of patients were based on self-reported data which might be subject to various biases such as reactivity or selective-reporting bias. Given the limited financial resources, it was not possible to compare the effect of probiotics and vitamin D co-supplementations on migraine features to that of probiotics and vitamin D alone. Finally, we did not gather information regarding the type of migraine headache (episodic or chronic); thus, it was not possible to evaluate the efficacy of probiotic and vitamin D co-supplementation by the type of migraine headache.

Conclusions

In conclusion, we found that probiotic and vitamin D co-supplementation may have beneficial effects on migraine headache frequency and severity, but did not affect migraine headache duration, and serum levels of hs-CRP. Further research is required to find alternative treatment options for the management of mental health problems in migraineurs.

Supplementary information.

Abbreviations

ANCOVA	Analysis of covariance
BMI	Body mass index
BP	Blood pressure
CFU	Colony forming unit
CM	Chronic migraine
CVS	Cyclic vomiting syndrome
DASS	Depression, anxiety, stress scale
DBP	Diastolic blood pressure
DGBI	Disorders of gut-brain interaction
EM	Episodic migraine
HIT-6	Headache Impact Test-6
hs-CRP	High-sensitivity C-reactive protein
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
ICHD-3	International Classification of Headache Disorders
IL	Interleukin
ITT	Intention-to-treat
GBD	Global Burden of Disease
GI	Gastrointestinal
MAP	Mean arterial pressure
MET.hr/d	Metabolic Equivalent hours per day
QoL	Quality of life
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SNRIs	Serotonin and norepinephrine reuptake inhibitors
TCAs	Tricyclic antidepressants
TNF-α	Tumor necrosis factor-alpha
VAS	Visual analog scale
YLDs	Years living with disability

Supplementary Information

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Additional file 1. Table S1. Effects of probiotic and vitamin D co-supplementation on migraine symptoms and HIT-6 score based on per protocol analyses. Table S2. Effects of probiotic and vitamin D co-supplementation on depression, anxiety, and depression based on per protocol analyses.

Additional file 2.

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Authors' contribution
SAT, FK, PS, ZM, GA contributed in conception, design, data collection, data interpretation, manuscript drafting, approval of the final version of the manuscript, and agreed for all aspects of the work.

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Data Availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The Ethics Committee of Isfahan University of Medical Sciences approved the study protocol (no.3401664) and a written informed consent form was signed by each patient before study initiation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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