European Journal of Nutrition (2025) 64:179
https://doi.org/10.1007/s00394-025-03687-w

ORIGINAL CONTRIBUTION

Effects of probiotic and vitamin D co-supplementation on migraine index, quality of life, and oxidative stress in adults with migraine headache: a randomized triple-blinded clinical trial

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Received: 24 October 2024 / Accepted: 11 April 2025 / Published online: 13 May 2025

Abstract

Background The current study was conducted to assess the effect of probiotic and vitamin D co-supplementation on migraine index (MI), migraine-specific quality of life (MSQoL), and oxidative stress in adults with migraine.

Methods This parallel randomized, triple-blinded, placebo-controlled trial was conducted among adult individuals aged 18 to 55 years with a diagnosis of migraine headache according to the International Classification of Headache Disorders-3 (ICHD-3). Patients were randomized to either multispecies probiotic (4.5×10^{11} CFU per day) plus vitamin D (50,000 IU every two weeks) or placebo for 12 weeks. MI, MSQoL, blood pressure, anthropometric, and biochemical variables (25-hydroxy vitamin D, nitric oxide (NO), malondialdehyde (MDA), total oxidative status (TOS), total antioxidant capacity (TAC), and catalase (CAT)) were examined at baseline and after 12 weeks of intervention.

Results In total, 72 patients (36 patients in each group) with migraine headache were included in the study. The mean age of patients was 37.46 ± 0.98 and sixty five out of 72 patients were females. A significant increase in mean serum levels of 25-hydroxy vitamin D was observed in the probiotic plus vitamin D group compared to the placebo group ($p < 0.001$). A significantly greater reduction in mean MI (-30.11 ± 6.95 vs. -11.97 ± 3.05; $p = 0.01$) was found in the probiotic plus vitamin D than in the placebo group. In addition, a marginally significant difference was observed between the two groups regarding changes in serum levels of NO ($p = 0.05$).

Conclusion This trial showed that probiotic plus vitamin D supplementation can favorably improve MI as well as serum levels of NO in adult patients with migraine. Further research on mechanisms through which the supplementation was effective on MI is warranted.

Keywords Probiotic · Vitamin D · Migraine index · Oxidative stress · Migraine-specific quality of life

Abbreviations

ANCOVA	Analysis of covariance
BMI	Body mass index
BP	Blood pressure
CAT	Catalase
CFU	Colony forming unit
DBP	Diastolic blood pressure
EF	Emotional function
HRQoL	Health-related quality of life
ICHD-3	International Classification of Headache Disorders
ITT	Intention-to-treat
MAP	Mean arterial pressure
IU	International unit
MDA	Malondialdehyde
MET.hr/d	Metabolic equivalent hours per day
MI	Migraine index
MSQoL	Migraine-specific quality of life
NO	Nitric oxide
ROS	Reactive oxygen species
RP	Role function-preventive
RR	Role function-restrictive
SBP	Systolic blood pressure

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SD	Standard deviation
SE	Standard error
TAC	Total antioxidant capacity
TCAs	Tricyclic antidepressants
TOS	Total oxidative status
VAS	Visual analogue scale

Introduction

Headache disorders are counted as top causes of years lived with disability based on the reports of Global Burden of Disease, 2019 [1]. Migraine is known as a common disabling primary headache with a prevalence of 15% per year worldwide. Clinically, migraine is characterized as recurrent headaches with other correlated symptoms such as nausea, vomiting, photophobia, and phonophobia [2]. Migraine is a life-long disease that substantially affects the quality of life and daily functioning of patients [3, 4]. Despite advances in treatment strategies, acute treatment of migraine headache remains suboptimal, which increases the risk of chronicity and medication overuse in patients [5, 6]. Therefore, discovering evidence-based strategies for the management of migraine headaches is essential.

The pathophysiological mechanisms of migraine headaches are not fully understood; however, some possible explanations have been proposed regarding the initiation and maintenance of migraine headaches. Reactive oxygen species (ROS) are synthesized as a result of natural metabolic and physiological processes in the body [7]. An imbalance between ROS synthesis and their elimination by the antioxidant system, which is known as oxidative stress, has been implied as a possible mechanism in migraine pathogenesis [8–12]. The majority of previous studies have shown an increase in the level of total oxidative status (TOS), nitric oxide (NO), and lipid peroxidation markers such as malondialdehyde (MDA) as well as a decrease in total antioxidant capacity (TAC) in patients with migraine [13]. Thus, a promising strategy for migraine treatment could be targeting oxidative stress in these patients [11, 13].

The WHO/FAO has defined probiotics as live microbial organisms that confer a beneficial effect on the host when administered in adequate amounts [14]. Their beneficial effects are their viability in the gastrointestinal tract, interaction with gut microbiota, and exertion of a range of biological activities such as immune modulation and reduction of oxidative stress [15]. One of the proposed mechanisms underlying their health benefits is their antioxidant properties [16]. Several previous meta-analyses and systematic reviews of randomized controlled trials have investigated the effect of probiotic supplementation on biomarkers of oxidative stress [17–22]. The findings of these studies have demonstrated the beneficial effect of probiotics on some biomarkers of oxidative stress in various subjects, like patients with chronic kidney disease, diabetes mellitus, psychological, and neurological disorders. However, there were some differences between the findings of these studies [17–22]. For example, although probiotic supplementation improved some oxidative stress biomarkers such as TAC, glutathione (GSH), and NO in patients with chronic kidney disease and diabetes mellitus [17, 18], it had no significant effect on any of these biomarkers in patients with psychological disorders [20]. In addition, it has been proposed that vitamin D has antioxidant properties and its impact on oxidative stress has received considerable attention in clinical research. However, findings obtained from prior studies were controversial [23–26]. For instance, it has been reported that vitamin D supplementation has a favorable effect on MDA and NO in diabetic patients [25]; nevertheless, it did not affect these biomarkers in patients with psychiatric disorders [23]. Therefore, it is impossible to draw a general conclusion and generalize the results of these studies to other patient populations.

Several previous studies have evaluated the effect of probiotic [27, 28] or vitamin D [29–31] supplementation on migraine headache; however, their findings were controversial. For example, an interventional study reported beneficial effects of probiotic supplementation on clinical features of migraine such as severity and frequency of headaches [27]. However, another study did not report a significant effect of probiotics supplementation on migraine headaches [28]. Furthermore, there were some differences between the findings of various studies investigating the impact of vitamin D supplementation on migraine headaches. The findings of a trial indicated that the administration of 2000 IU vitamin D was effective on the severity, frequency, and duration of headaches [30], while other trials that used vitamin D supplements in doses of 50,000 IU or 4000 IU reported only favorable effects on the frequency of headaches [29, 31]. In addition, previous studies of both vitamin D and probiotic supplementation have not examined changes in biomarkers of oxidative stress and NO as potential mediators of migraine headaches. Regarding favorable effects of probiotic and vitamin D supplementation on migraine headaches as well as available evidence on the synergic effect of probiotic and vitamin D [32], we hypothesized that probiotic and vitamin D co-supplementation may favorably impact migraine headaches in adult patients. To enhance patient compliance and increase serum vitamin D levels over a 12-week period, a dose of 50,000 IU of vitamin D every 2 weeks was administered in this study. Additionally, based on previous evidence supporting the effects of multi-species probiotics on migraine headaches as well as benefiting from different bacterial strains in these supplements, multi-species probiotics were incorporated into this study. Thus, the present randomized triple-blinded clinical trial aimed to investigate

the effect of multispecies probiotic and vitamin D co-supplementation on migraine index (MI), migraine-specific quality of life (MSQoL), and some biomarkers of oxidative stress in patients with migraine.

Method

Study design and patients

This parallel randomized, triple-blinded, placebo-controlled trial was undertaken in a central neurology clinic in Isfahan, Iran. The protocol of the study was registered at the Iranian Registry of Clinical Trials (no. IRCT20121216011763N59; Registered 4 May 2023). The Ethics Committee of Isfahan University of Medical Sciences also approved the study protocol (no. 3401664). Objectives of the study were explained to all patients and written informed consent was obtained. The minimum sample size was estimated to be 35 patients in each group using the mean difference and standard deviation for NO, as a key outcome, according to a previous study considering a power of 90% and type I error of 0.05 $((z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \sigma^2 / \mu_1 - \mu_2)$ [33]. Eligible patients were aged 18–55 years old, diagnosed with migraine headaches (with or without aura) based on the International Classification of Headache Disorders-3 (ICHD-3) by a neurologist [2], and experienced at least 2 migraine attacks per month during the 3 previous months. Pregnant or breast-feeding women, drug-overuse headache (chronic headache due to frequent use of analgesics or anti-migraine drugs) [2], and those with clinical diagnosis of neurological, cardiovascular, endocrine, hepatic, renal, and gastrointestinal diseases were not included in the study. Taking vitamin D and probiotics supplements, antibiotic use, and probiotic-fortified foods consumption up to 3 months before the initiation of the study were considered as non-inclusion criteria, as well. The exclusion criteria were any modification in patients' treatment approach, adherence rate lower than 80%, and patients' refusal to continue the intervention.

Randomization and intervention

Patients were recruited from May 2023 to October 2023. A list created by a web-based system (https://www.sealedenvelope.com/) using a permuted block with a size of four (stratifies based on age and sex) was used to assign patients randomly to the probiotic plus vitamin D supplementation group or placebo group by an independent investigator. Patients in the intervention group were treated with a gelatine multispecies probiotic capsule (containing 8 species of *Lactobacillus plantarum* BP06, *Lactobacillus casei* BP07, *Lactobacillus acidophilus* BA05, *Lactobacillus bulgaricus* BD08, *Bifidobacterium infantis* BI04, *Bifidobacterium longum* BL03, *Bifidobacterium breve* BB02, and *Streptococcus thermophilus* BT01; 4.5×10^{11} CFU) (Fara Daru Fanavaran Mehr (Farabiotic) Pharmaceutical Company, Tehran, Iran) every day before lunch and a pearl of vitamin D (50,000 IU) (Zahravi Pharmaceutical Company, Tabriz, Iran) every two weeks with launch for 12 weeks. Patients in the placebo group were also instructed to consume a placebo capsule for probiotic, containing 330 mg starch and maltodextrin in equal amounts, every day and a placebo pearl, containing 100 mg corn oil, for vitamin D every 2 weeks for 12 weeks. Supplement and placebo capsules (or pearls) were the same in shape, color, size, and packaging. All patients, investigators, and analyzers in the two groups were blinded to the treatment and did not have any access to randomization list and the type of administered supplements.

Migraine headache assessment

A one-month headache diary was provided to the patients to record information in terms of headache severity (on a 10-cm visual analog scale (VAS)), duration (hour), and frequency of attacks per month. Headache diaries were completed 4 weeks before the intervention and in the last 4 weeks of the intervention. The collected data were used to calculate MI (frequency × severity) [34].

Biochemical variables

Blood samples were collected in tubes after 12 h of fasting at baseline and 12 weeks after the intervention in a medical library. After centrifuging at 3500 rpm for 10 min, the 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 with interassay and intraassay CV % less than 10% (DiaZist company, Tehran, Iran). Serum levels of NO, MDA, TAC, TOS, and catalase (CAT) were examined by an available commercial kit with inter-assay CV% less than 10% and intra-assay CV% less than 12% (Kiazist Life Sciences, Iran). All biochemical variables were measured twice both at baseline and after 12 weeks of intervention and the average of values was used for statistical analyses.

Blood pressure and anthropometric variables

BP and anthropometric variables were measured at baseline and after 12 weeks of the intervention. Blood pressure (BP) was measured two times, five minutes apart, using a digital sphygmomanometer (Rossmax Swiss GmbH, Heerbrugg, Switzerland) in a sitting position after a 10-min resting. The mean of two measurements was used for statistical analysis. Mean arterial pressure (MAP) was approximated using the

following formula: [2 diastolic blood pressure (DBP) + systolic blood pressure (SBP)]/3] [35]. A trained dietitian evaluated anthropometric parameters while subjects were standing in light clothing with no shoes. Weight (kg) was measured to the nearest 0.1 kg by a digital scale (Omron, HN-286, Kyoto, Japan) and height (cm) was measured to the nearest 0.1 cm by a wall-mounted tape measure. Body mass index (BMI) (kg/m^2) was computed by dividing weight by the height squared.

Other variables

In the first face-to-face visit, data on patients' age, sex, educational status, marital status, family history of migraine headache, medication use, smoking, education, and menopausal status were collected by a standard checklist. Patients were also instructed by a trained dietitian to record their dietary intakes after 1 week of the intervention and every 2 weeks during 12 weeks of intervention. Household measures were used to convert reported portion sizes of consumed foods to grams per day and data inserted in the Nutritionist IV software (N-Squared Computing, Salem, OR, USA). Then, the mean daily intake of energy and nutrients was estimated using the Nutritionist IV software. Patients were instructed by a trained dietitian to record their physical activity on two non-consecutive days (to represent natural variations in physical activity) during the intervention and the data were converted to Metabolic Equivalent Task hours per day (MET.hr/d) using the formula of Σ ((MET × min)/day).

Health-related QoL was evaluated at baseline and after 12 weeks of the intervention using a validated MSQoL questionnaire [36]. This self-administered tool assesses the effect of migraine headaches on Health-related QoL in three domains: role function-restrictive (RR) (7 items evaluating the functional effect of migraine headaches on social activities and daily work), role function-preventive (RP) (4 items evaluating the effect of migraine headache on social activities and daily work prevention), and emotional function (EF) (3 items evaluating the emotional effect of migraine headaches). Items in each domain were responded on a 6-point Likert scale ranging from 0 (never) to 6 (all the time). The total raw score was obtained by summing the score of each item and rescaled to a score ranging from 0 to 100. Higher scores represented higher MSQoL.

Statistical analysis

Statistical analyses were performed using SPSS software version 20 (IBM, Chicago, IL) and a P-value < 0.05 (two-tailed) was considered statistically significant. Normality of continuous variables was evaluated using Kolmogorov–Smirnov test. Quantitative and categorical variables were reported as mean (SE) and frequency (percentage), respectively. Comparison of baseline characteristics was performed using an independent samples *t*-test (age, weight, BMI, MAP, and serum levels of 25-hydroxy vitamin D) or a Pearson's chi-square test (sex, smoking, education, marital status, menopausal status, family history of migraine, migraine with aura, and medications). A paired sample *t*-test was used to assess within-group modifications of variables during the intervention. Independent samples *t*-test and analysis of covariance (ANCOVA) were used to evaluate differences in investigated outcomes between probiotic plus vitamin D and placebo groups. The confounders, including taking tricyclic antidepressants (TCAs), taking triptans, and baseline MAP were controlled in the ANCOVA test. Both per-protocol and intention-to-treat (ITT) analyses were done. The last-observation-carried-forward method was applied to treat missing values in ITT analyses.

Results

In total, 72 patients (comprising 90.3% females and 9.7% males) were randomized to probiotic plus vitamin D ($n = 36$) or placebo ($n = 36$); 68 of them completed the trial ($n_{\text{probiotic plus vitamin D group}} = 34$; $n_{\text{placebo group}} = 34$) which were included in the pre-protocol analysis. Four patients were excluded from the study because of reluctance to continue the intervention due to a lack of interest ($n = 3$) and poor compliance with the treatment ($n = 1$), as depicted in Fig. 1. ITT analysis was also done for all included patients by replacing the missing data with the last observed value.

The mean age and BMI of patients were 37.46 years and 25.04 kg/m^2, respectively. The mean frequency, duration, and severity of migraine headaches in the total sample were 6.82 ± 0.53 attacks per month, 23.69 ± 2.54 h, and 8.33 ± 0.18 (based on VAS scale), respectively. MAP (86.97 ± 1.36 vs 91.08 ± 1.09 mmHg; $p = 0.02$) was significantly lower in the probiotic plus vitamin D group than the placebo group. The frequency of patients treated with triptans (placebo: 16.7%; probiotic plus vitamin D: 41.7%; $p = 0.04$) and tricyclic antidepressants (TCAs) (placebo: 69.4%; probiotic plus vitamin D: 41.7%; $p = 0.03$) was also different between the two groups. However, there was not any significant difference between the two arms of the intervention regarding other basic variables (Table 1).

The comparison of dietary intakes of participants through the intervention indicated no significant difference between probiotic plus vitamin D and placebo groups regarding mean dietary intakes of energy, macronutrients, magnesium, potassium, calcium, vitamin C, vitamin E, vitamin A, and folate (Supplementary Table 1). As shown in Fig. 2A, no significant difference in mean physical activity during the intervention was also observed between probiotic plus vitamin D and placebo groups ($p = 0.41$). Mean weight

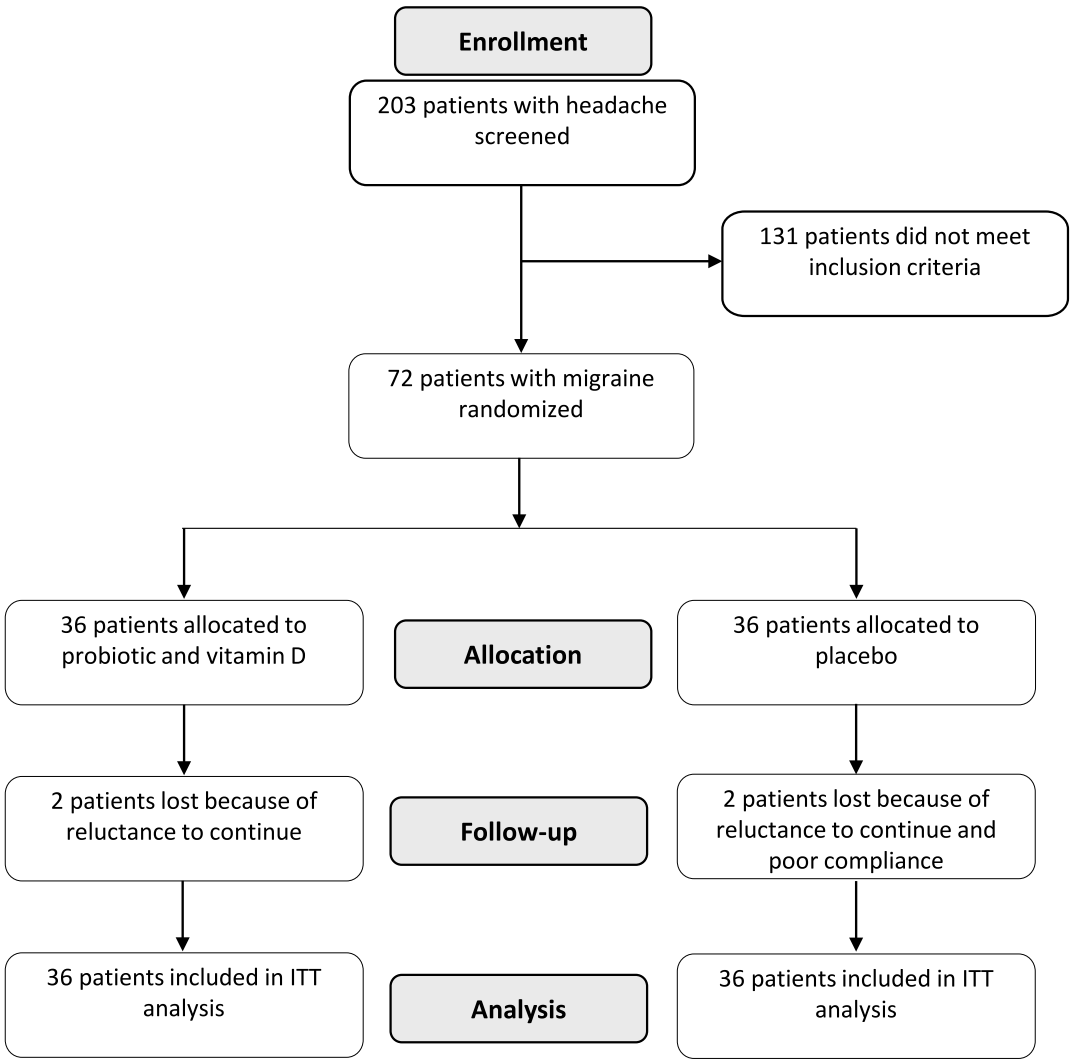

Fig. 1 Patients' flow diagram

and BMI changes in probiotic plus vitamin D and placebo groups after 12 weeks of intervention have been presented in Fig. 2B, C. No significant difference was observed between the two arms of the intervention regarding changes in weight ($p=0.54$) and BMI ($p=0.49$).

The mean 25-hydroxy vitamin D after 12 weeks of intervention was 31.05 ± 2.01 ng/ml in the probiotic plus vitamin D (mean change from baseline value: $+12.86 \pm 1.64$ ng/ml) and 18.89 ± 1.24 in the placebo group (mean change from baseline value: $+1.12 \pm 0.80$ ng/mL) ($P_{\text{between intervention and placebo}} < 0.001$).

Table 2 presents the effect of the intervention on MI and MSQoL based on ITT analyses. Within-group analyses indicated that mean MI decreased significantly both in the probiotic plus vitamin D (32.67 ± 4.41 vs. 62.78 ± 6.80; $p<0.001$) and placebo (38.86 ± 6.35 vs 50.83 ± 6.29; $p<0.001$) groups compared to the baseline values. In addition, the mean MSQoL was significantly increased in both probiotic plus vitamin D (66.67 ± 2.41 vs 55.75 ± 3.24; $p<0.001$) and placebo (62.43 ± 2.70 vs 55.29 ± 2.99; $p=0.01$) arms of the intervention. The results of between-group analyses showed significant reductions in mean MI (-30.11 ± 6.95 vs -11.97 ± 3.05; $p=0.02$) in the probiotic plus vitamin D versus placebo group. In terms of MSQoL score, mean changes between the probiotic plus vitamin D and placebo groups were not statistically significant (10.91 ± 2.55 vs. 7.14 ± 2.37; $p=0.28$). Analysis of covariance indicated a significant difference in mean MI reduction between two intervention arms ($p=0.01$). However, there was no significant differences in MSQoL score ($p=0.29$) changes between two groups. Similar findings were obtained from per-protocol analyses, as shown in Supplementary Table 2.

Table 3 presents the effect of the probiotic plus vitamin D intervention on oxidative stress biomarkers based on ITT

Table 1 Basic characteristics of study participants in probiotic plus vitamin D and placebo groups ($n=72$)

Variables	Probiotic plus vitamin D ($n=36$)	Placebo ($n=36$)	p-Value*
Age (year)	37.44 ± 1.47	37.47 ± 1.32	0.99
Females	33 (91.7)	32 (88.9)	0.99
Body weight (kg)	67.81 ± 1.84	67.32 ± 1.83	0.85
Body mass index (kg/m^2)	24.97 ± 0.51	24.89 ± 0.59	0.91
Smoking (yes)	2 (5.6)	0 (0.0)	0.31
Education (year)			
≤ 12 years	17 (47.2)	20 (55.6)	0.64
> 12 years	19 (52.8)	16 (44.4)	
Marital status			
Single	7 (19.4)	9 (25.0)	0.73
Married	27 (75.0)	24 (66.7)	
Previously married	2 (5.6)	3 (8.3)	
Mean arterial pressure	86.97 ± 1.36	91.08 ± 1.09	**0.02**
Postmenopausal females	2 (5.6)	4 (11.1)	0.67
Family history of migraine	8 (22.2)	9 (25.0)	0.99
Migraine with aura	14 (38.9)	21 (58.3)	0.16
MI at baseline	62.78 ± 6.79	50.83 ± 6.28	0.20
25(OH) vitamin D (ng/ml)	18.06 ± 1.14	17.97 ± 1.21	0.96
Medications			
Tricyclic antidepressants (TCAs)	15 (41.7)	25 (69.4)	**0.03**
Beta-blockers	13 (36.1)	12 (33.3)	0.99
Triptans	15 (41.7)	6 (16.7)	**0.04**
Serotonin and norepinephrine reuptake inhibitors (SNRIs)	4 (11.1)	3 (8.3)	0.99
Benzodiazepines	4 (11.1)	3 (8.3)	0.99
Others	7 (19.44)	6 (16.67)	0.99

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

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

All bold values are significantly different between probiotic plus vitamin D and placebo groups. ($p<0.05$ was considered statistically significant)

Table 2 Effects of probiotic and vitamin D co-supplementation on migraine index and migrain-specific quality of life (based on intention to treat analyses, $n=72$)*

Variables	Intervention ($n=36$)	Placebo ($n=36$)	Mean difference	p-Value
MI (frequency × severity)				
Baseline	62.78 ± 6.80	50.83 ± 6.29	−18.14 ± 7.59[b]	**0.02**[b]
12th week	32.67 ± 4.41	38.86 ± 6.35	[−33.40, −2.88]	**0.01**[c]
Mean change	−30.11 ± 6.95	−11.97 ± 3.05		
p-Value	**< 0.001**[a]	**< 0.001**[a]		
MSQoL score				
Baseline	55.75 ± 3.24	55.29 ± 2.99	− 3.77 ± 3.49[b]	0.28[b]
12th week	66.67 ± 2.41	62.43 ± 2.70	[−3.18, 10.72]	0.29[c]
Mean change	10.91 ± 2.55	7.14 ± 2.37		
p-Value	**< 0.001**[a]	**< 0.01**[a]		

*All values are presented as Mean ± SE

[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

MI migraine index; *MSQoL* migraine-specific quality of life

Table 3 Effects of probiotic and vitamin D co-supplementation on oxidative stress markers (based on intention to treat analyses, $n=72$)*

Variables	Probiotic plus vitamin D ($n=36$)	Placebo ($n=36$)	Mean difference	*p*-Value
MDA (nmol/mL)				
Baseline	53.06 ± 3.22	49.10 ± 3.34	− 6.72 ± 5.69[b] [−18.06, 4.63]	0.24[b] 0.69[b]
12th week	44.17 ± 1.68	48.77 ± 3.42		
Mean change	−8.89 ± 3.71	−2.17 ± 4.31		
p-Value	**0.02**[a]	0.62[a]		
NO (nmol/mL)				
Baseline	39.21 ± 1.07	36.83 ± 1.01	− 4.49 ± 2.15[b] [−8.78, −0.19]	**0.04**[b] **0.05**[c]
12th week	35.42 ± 0.89	37.52 ± 1.80		
Mean change	−3.80 ± 1.18	0.69 ± 1.80		
p-Value	**0.01**[a]	0.71[a]		
TOS (nmol/mL)				
Baseline	13.97 ± 0.69	13.93 ± 0.83	−0.53 ± 1.56[b] [−3.64, 2.57]	0.73[b] 0.64[c]
12th week	11.33 ± 0.74	10.75 ± 0.78		
Mean change	−2.64 ± 0.91	−3.17 ± 1.27		
p-Value	**0.01**[a]	**0.02**[a]		
TAC (nmol/mL)				
Baseline	1233.49 ± 53.49	1191.85 ± 77.86	136.96 ± 117.69[b] [−97.77, 371.69]	0.25[b] 0.08[c]
12th week	1394.00 ± 60.66	1168.31 ± 60.70		
Mean change	160.51 ± 73.20	23.55 ± 92.16		
p-Value	**0.03**[a]	0.80[a]		
CAT (nmol/min/mL)				
Baseline	66.41 ± 3.88	68.24 ± 5.55	6.59 ± 6.23[b] [−5.83, 19.01]	0.29[b] 0.19[c]
12th week	76.06 ± 4.10	71.30 ± 3.91		
Mean change	9.66 ± 3.80	3.07 ± 4.93		
p-Value	**0.02**[a]	0.54[a]		

*All values are presented as Mean ± SE

[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

MDA malondialdehyde; *NO* nitric oxide; *TOS* total oxidant status; *TAC* total antioxidant capacity; *CAT* catalase

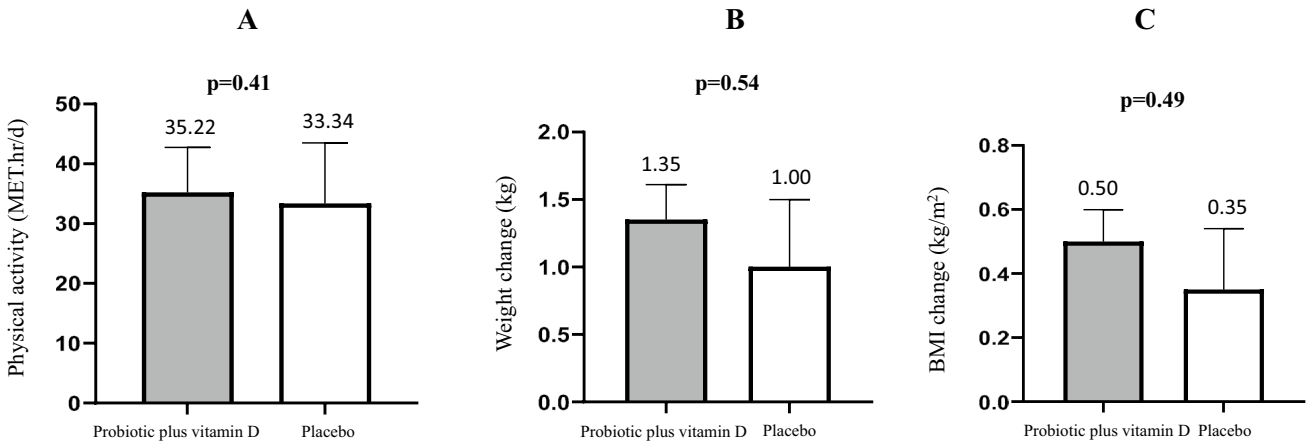

Fig. 2 The comparison of physical activity level (**A**), changes in weight (**B**) and BMI between probiotic plus vitamin D and placebo groups

analyses. Within-group analyses indicated that mean serum levels of NO, MDA, and TOS decreased significantly in the probiotic plus vitamin D group compared to baseline ($p < 0.05$). However, mean serum levels of CAT and TAC increased significantly in the probiotic plus vitamin D group after 12 weeks of the intervention ($p < 0.05$). No significant difference was observed in mean serum levels of NO, MDA, CAT, and TAC after 12 weeks in the placebo group compared to the baseline values ($p > 0.05$). After 12 weeks of the intervention, the mean serum levels of TOS decreased significantly in the placebo group ($p = 0.02$). Between-group analyses showed a significant reduction in mean serum levels of NO in the probiotic plus vitamin D compared to the placebo group (-3.80 ± 1.18 vs 0.69 ± 1.80; $p = 0.04$). However, no significant difference was observed in mean changes of serum levels of MDA (-8.89 ± 3.71 vs -2.17 ± 4.31; $p = 0.24$), TOS (-2.64 ± 0.91 vs -3.17 ± 1.27; $p = 0.73$), TAC (160.51 ± 73.20 vs 23.55 ± 92.16; $p = 0.25$), and CAT (9.66 ± 3.80 vs 3.07 ± 4.93; $p = 0.29$) in the probiotic plus vitamin D group compared to placebo group. After taking the confounding variables into account a marginally significant difference was observed between the two groups in mean serum levels of NO ($p = 0.05$); however, no significant difference was observed between the two intervention arms regarding serum levels of MDA ($p = 0.69$), TOS ($p = 0.64$), TAC ($p = 0.09$, and CAT ($p = 0.19$). Similar findings were obtained from per-protocol analyses on 68 patients, except a significant between-group difference in mean serum levels of NO, after adjustments for confounders ($p = 0.04$) (Supplementary Table 3). No adverse effect related to the intervention was reported in the two groups.

Discussion

Emerging evidence suggests that the gut-brain axis, the bidirectional link between the gastrointestinal tract and the central nervous system, is associated with various psychological and neurological disorders such as depression, anxiety, stress, Parkinson's disease, Alzheimer's disease, and autism [37–39]. Furthermore, current data also indicates that the gut–brain axis affects migraine etiology; however, underlying mechanisms are not determined completely [40]. Therefore, therapeutic approaches that modulate gut microbiota seem advantageous for preventing and treating conditions related to the gut–brain axis such as migraine headaches.

The present investigation found that probiotic and vitamin D co-supplementation for 12 weeks improved MI in adult patients with migraine. The evaluation of the supplementation on some biomarkers of oxidative stress showed a marginally significant difference between the two arms of intervention regarding changes in serum levels of NO. However, we did not find any significant effect of the treatment on MSQoL and other biomarkers of oxidative stress.

Few previous studies have investigated the influence of probiotic supplementation on clinical characteristics of patients with migraine headache [27, 28]. A prior intervention by Martami et al. involving 79 Iranian patients reported that administration of two multi-species probiotic capsules at a total dose of 5×10^9 CFU (containing 14 bacterial strains of *Bacillus subtilis* PXN21, *Bifidobacterium bifidum* PXN 23, *Bifidobacterium breve* PXN25, *Bifidobacterium infantis* PXN27, *Bifidobacterium longum* PXN30, *Lactobacillus acidophilus* PXN35, *Lactob. delbrueckii ssp. Bulgaricus* PXN39, *Lactob. Casei* PXN37, *Lactob. Plantarum* PXN47, *Lactob. Rhamnosus* PXN54, *Lactob. Helveticus* PXN45, *Lactob. Salivarius* PXN57, *Lactococcus lactis ssp. Lactis* PXN63, and *Streptococcus thermophilus* PXN66), for 10 weeks in patients with episodic migraine and 8 weeks in patients with chronic migraine, reduced migraine headache frequency and severity, as well as the number of taken abortive drugs per day, both in patients with episodic and chronic migraine [27]. However, a previous randomized placebo-controlled trial conducted in the Netherlands by Ross et al. on 63 patients with episodic migraine headache showed that consumption of a multi-species probiotic supplementation (containing 8 bacterial strains of *Bifidobacterium bifidum* W23, *B. lactis* W52, *Lactobacillus acidophilus* W37, *Lactob. Brevis* W63, *Lactob. Casei* W56, *Lactob. salivarius* W24, *Lactococcus lactis* W19, and *Lactoc. Lactis* 58) with a dosage of 5×10^9 CFU for 12 weeks resulted in no significant difference in migraine headache frequency and severity compared to the placebo group [28]. In the present study, probiotic and vitamin D co-supplementation improved MI, as a representative of migraine headache frequency and severity, after 12 weeks. Different findings of the study conducted by Ross et al. might be related to different type of bacterial strains and genus in the administered probiotic supplement, supplementation dose, and studied subjects. For example, the dosage of administered probiotic supplement in the study by De Roos et al. was lower than the administered probiotic supplement in the current study and its effects was only investigated in patients with episodic migraine. Additionally, the bacterial combination of probiotic supplement in the study by De Roos et al. was different from the administered supplement in the current study regarding their genus and strain. We also assume that considering the heterogeneity of gut microbiota in diverse races and populations [41, 42], the response to probiotic supplementation might probably be different in patients from various communities; however, this assumption needs further assessment. Considering these controversial findings of previous studies, further research is needed to explore the optimal probiotic mixture for the treatment of patients with various subtypes of migraine headache. Furthermore, it is

important to investigate whether the effects of this supplement in migraine patients are dose-dependent.

Similar to our findings, the favorable effect of vitamin D supplementation on some clinical features of migraine headache has been reported by several previous randomized trials. The administration of 100 µg (4000 IU) vitamin D per day for 24 weeks resulted in a significant decrease in migraine frequency compared to placebo. However, this supplementation had no significant effect on other investigated variables such as migraine-related symptoms, headache severity, and headache impact test-6 scores [29]. Mottaghi et al. evaluated the effect of vitamin D supplementation (a pearl of 50,000 IU/week) in 65 Iranian patients with migraine headache (aged 10–61 years) for 10 weeks. The findings of the study also indicated that vitamin D supplementation decreased migraine headache frequency [31]. Evaluation of the effect of 2000 IU vitamin D per day for 12 weeks by Ghorbani et al. also showed promising results on clinical characteristics of migraine headache comprising frequency, severity, and attacks duration in patients with episodic migraine [30].

Although probiotic plus vitamin D supplementation improved MI in patients with migraine headaches in the present study, no significant effect on MSQoL was observed. We postulated that other factors besides headaches' frequency and severity may affect QoL in patients with migraine headache. For example, the findings of previous research have indicated that the prevalence of psychiatric disorders such as anxiety and depression is higher in migraineurs which may have an unfavorable effect on QoL in these patients [43, 44]. Thus, to improve QoL in patients with migraine headaches effectively, it is perhaps necessary to use a combination therapy approach comprising both pharmacotherapy and cognitive behavioral therapy.

Many studies have demonstrated the possible role of oxidative stress in the pathogenesis of migraine headache [13]. However, there is insufficient information on the effect of prophylactic treatments on oxidative indices in patients with migraine. On contrary to our findings, two preceding randomized controlled trials conducted on women with gestational diabetes and polycystic ovary syndrome showed that probiotic and vitamin D co-supplementation had no significant effect on serum levels of NO [45, 46]. Compared with these studies, serum NO levels were higher in patients with migraine in the present study, which may partially explain the significant reduction in NO levels in migraine patients after treatment with probiotic and vitamin D supplements. This assumption is affirmed by previous evidence on the higher levels of NO during migraine attacks and interictal periods [47–49]. For example, a study has indicated that patients with migraine experience an increase in cyclic guanosine monophosphate (cGMP), as the secondary messenger in NO signaling pathway, during migraine attacks [47]. Additionally, higher levels of NO in patients with migraine compared to controls during headache-free periods have been demonstrated by other studies [48, 49]. To explore the effect of probiotic and vitamin D co-supplementation on other oxidative biomarkers in patients with migraine, further studies with a longer follow up duration on a larger sample size are recommended.

Probiotic and vitamin D co-supplementation is probably effective on migraine headache in adult patients through NO synthesis inhibition. There are some indications regarding the role of NO in migraine headache initiation and maintenance. Such that, the administration of nitroglycerine, as a NO donor, induces mild headaches in healthy subjects and severe persistent headaches in subjects with migraine [50]. Some evidence has implied the potential influence of vitamin D on NO levels. For example, a previous research has indicated that there is an inverse association between NO and serum levels of vitamin D in adolescents [51]. The result of a previous in vitro study has also indicated that vitamin D can prevent oxidative damage to hippocampal neuron cultures by suppressing the expression of iNOS mRNA [52]. Some mechanisms can explain the effect of probiotics on migraine headache through reducing NO synthesis. It has been demonstrated that short-chain fatty acids (acetate, propionate, and butyrate) produced by probiotic bacteria such as *Lactobasillus* and *Bifidobacterium* attenuate the production of NO which plays a crucial role in migraine pathogenesis [53–55]. Furthermore, probiotics exert anti-endotoxemia properties by modulating gut microbiota which resulted in oxidative stress modulation [56]. Some evidence has shown synergic effect of probiotics and vitamin D. It has been suggested that probiotics increase the expression of vitamin D receptor and rise the intestinal absorption of vitamin D [32]. It seems that vitamin D receptor plays a key role in anti-inflammatory effects of probiotics [57, 58]. However, further studies are required to detect underlying mechanisms of the interaction between vitamin D and probiotics.

The present study has some strengths and limitations that should be addressed. The study was the first randomized controlled trials that investigated the impact of probiotic and vitamin D co-supplementation on oxidative stress biomarkers in adult patients with migraine headache. Furthermore, the adherence of patients to the treatment, presented by serum levels of 25-hydroxy vitamin D, was high. However, the generalizability of our findings to male patients with migraine is limited, because the majority of our patients were females. Due to limited budget, it was not possible to assess intestinal permeability, gut microbiota changes, and other biomarkers of oxidative stress to precisely explore the effect of the supplementation and underlying mechanisms on patients with migraine headache. In addition, it is unknown whether the beneficial effect of the prescribed supplementation is related to both components or the effect of their

combination is greater than the effect of their administration alone. Although the study subjects were randomized, significant differences were observed between the two groups in some basic characteristics, such as medication use. However, variables with significant differences between the two groups were controlled for in the statistical analysis. This study showed the favorable effect of probiotic and vitamin D co-supplementation on MI and some oxidative stress biomarkers as potential contributors of migraine headaches in adult patients; however, further studies are needed to compare the effects of vitamin D and probiotic supplements with their co-administered supplement on migraine headaches.

Conclusion

In summary, the results of the present study showed that probiotic and vitamin D co-supplementation had beneficial effects on MI and serum levels of NO. However, the treatment option did not significantly affect the quality of life and other oxidative stress biomarkers. Further studies are warranted to extend these investigations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00394-025-03687-w.

Acknowledgements The authors are grateful for the participation of all patients.

Author contributions SAT, PS, FK, and 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.

Funding This paper is based on the Master thesis approved by the Isfahan University of Medical Sciences, Isfahan, Iran.

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

Declarations

Conflicts of interest None of the authors had any personal or financial conflicts of interest.

Ethical approval The Ethics Committee of Isfahan University of Medical Sciences approved the study protocol (no.3401664).

Consent for publication Not applicable.

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