Title: Effect of Synbiotic Supplementation on Cognition and Activities of Daily Living (ADLs) in the Elderly with Alzheimer's Disease: A Randomized, Double-Blinded Trial

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Abstract

Background: Dementia is a progressive neurodegenerative disorder and a significant healthcare concern increasing worldwide. The modulation of the gut-brain axis by gut microbiota might have favorable effects on ameliorating cognitive decline. Our objective was to investigate whether synbiotics administration could enhance cognition and function in older adults with non-severe Alzheimer's disease (AD).

Methods: This study was designed as a randomized, placebo-controlled, double-blinded clinical trial to test the effects of synbiotic supplementation for 90 days (Between August 2019 and February 2021). A synbiotics formulation or placebo was randomly allocated to older outpatients with mild to moderate AD. The intervention group took two capsules daily containing the mixture of 7 bacterial strains as probiotics and a prebiotic for 12 weeks. Mini-Mental State Examination (MMSE), and Barthel index evaluated participants' cognition and functional status. Pre- and post-intervention fasting blood samples were obtained to compare the serum albumin, fasting blood sugar (FBS), 25(OH) vitamin D, and lipid profile.

Results: 60 patients (Mean age of 77 years) were recruited. After 12 weeks of synbiotic supplementation, no significant improvement was detected in the MMSE Score (p=0.53) and Barthel index (p=0.43), respectively. Furthermore, metabolic parameters including FBS (p=0.92), triglyceride (P=0.48), total cholesterol (P=0.74), high-density lipoprotein (P=0.54), low-density lipoprotein (P=0.79), serum albumin (p=0.28) and 25(OH) vitamin D levels (p=0.67) were not different before and after synbiotic administration.

Conclusion: This study does not support short-term synbiotic supplementation to enhance cognitive and physical function in elderly patients with mild to moderate AD.

Keywords: Synbiotics, Cognitive function, Activity of daily living, Elderly, Alzheimer's disease

Background

Dementia, as one of the foremost causes of mortality worldwide, is a neurodegenerative disorder characterized by progressive cognitive, behavioral, and functional decline (1-4). According to the World Alzheimer Reports, above 50 million individuals live with dementia throughout the world, and it is projected to affect almost 150 million people by 2050(5, 6). It could be associated with genetics, increasing age, low level of education, cardiovascular risk factors, and an unhealthy lifestyle(5). Oxidative stress, chronic neuroinflammation, and gut microbiota alternations could also be related to the pathophysiology of dementia. While dementia has a significant deleterious impact on patients' and their caregivers' quality of life and the substantial economic burden on the health care system, implanting practical preventive and therapeutic strategies and optimal care management is vital(7-9).

Recently published studies have demonstrated that the composition of gut microbiota and its bidirectional signaling pathways with the brain, called the gut-brain axis (GBA), might have a substantial role in the pathophysiology of neurocognitive disorders, including dementia(10-12). Consequently, enhancing beneficial bacteria in the gut by probiotics administration as a microbiome-based therapy could be advantageous for those patients through different pathways, including augmented short-chain fatty acid production, improved immune system function, diminished inflammation, and the level of oxidative stress (13-18). Although some evidence has shown that probiotics could raise physical and cognitive function(19, 20). Most studies are inconclusive with inconsistent results (21, 22) , and more research is needed to narrow this gap. The current study aimed to evaluate the effect of synbiotics on the cognition and physical function of older adults with dementia.

Methods

Study population

The 12-week randomized double-blinded trial participants were community-dwelling adults aged over 60 years who were recently diagnosed with mild to moderate dementia at the memory clinic of Ziaeian hospital, a medical and educational center affiliated with the Tehran University of Medical Sciences (TUMS). A neurologist and geriatrician have confirmed the diagnosis of dementia following the National Institute on Aging and Alzheimer's Association (NIA-AA) criteria(23). Only patients with Alzheimer's disease were selected for the study; regarding the wide range of symptoms in different types of dementia. Exclusion criteria were infection at the time of assessment, severe co-morbidities, gastrointestinal diseases or surgery, metabolic, immunosuppressive, and psychiatric disorders, alcohol or drug abuse, and taking antibiotics, probiotic supplements, or other drugs consumption of probiotic products in recent three months. Patients with any new conditions diminishing their cognition or physical function (e.g., delirium or stroke) over the study period or who were required to take antibiotics were also excluded. Accordingly, between August 2019 and February 2021, 60 patients were enrolled in the study.

Ethics Statements

The study was approved by the Ethics Committee of the Tehran University of Medical Science, code: (IR.TUMS.VCR.REC.1398.132) and the Iranian website for registration of the clinical trial :(IRCT 20191008045024N1)(24).

Informed consent was obtained from all participants' caregivers.

Study design and intervention

The standard formula for parallel clinical trials was used to calculate the sample size of the study. Based on previous studies (25) and considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%), the total number of participants needed is 30 in each group.

The random allocation sequence was accomplished through an online data center website(26). 30 participants were assigned to intervention and placebo groups by block randomization. All the patients received the medications for Alzheimer's disease and two capsules daily for 12 weeks.

Both synbiotics capsules (GeriLact ®) and placebos were produced by Zist Takhmir Company, Tehran, Iran. GeriLact ® is a gluten-free synbiotic (probiotic and prebiotic) formulation that contains 10^9 CFU of 7 bacterial strains, including Lactobacillus rhamnosus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium longum, and Streptococcus thermophilus plus fructooligosaccharides as prebiotic. The placebo capsules contained 500 mg maltodextrin and were identical to the synbiotics capsules in taste and physical appearance. All participants were asked to continue their routine lifestyle and not add any new nutritional supplements throughout the study period. Patients' adherence was monitored weekly during the study by weekly phone calls.

Data collection

Demographic data, including age, gender, education, occupation, dementia risk factors, co-morbidities, and medications, were recorded. A dietician educated caregivers to document dietary intakes by the 3-day-food record at the study's beginning and end. Dietary analysis was processed by nutritionist IV software (First Databank, San Bruno, CA)The cognition was evaluated at the

baseline and end of the study using the Persian version of the Mini-Mental State Examination (MMSE)(27), and functional status was measured using the Barthel index(28).

Fasting blood samples were collected to measure fasting blood sugar (FBS), triglycerides (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum albumin (Alb), and the serum level of 1,25(OH) vitamin D. Height was measured in meters using a wall tape in a standing position without shoes. Participants' weight (in kg) was assessed using a digital scale while minimally clothed at the study's onset and end. BMI was calculated as weight in kg divided by height in meters squared.

Statistical analysis

General characteristics of the study participants were reported using descriptive statistics (means, ± standard deviations (±SD), and percentages).

Kolmogorov-Smirnov test was applied to ensure the normal distribution of variables. Log-transformation was used in the case of variables with a non-normal distribution. Data on differences in macronutrient and micronutrient intakes between two groups were also compared using independent samples Student's t-test. Repeated measures analysis of variance (or its non-parametric equivalent) was applied to determine probiotic supplementations' effect on metabolic profiles, cognitive status, and functional performance. All statistical analyses were done using the Statistical Package for Social Science version 24 (SPSS Inc., Chicago, Illinois, USA), and p-values lower than 0.05 were considered statistically significant.

Results

Of 60 participants in the study, eight were excluded due to death or the occurrence of new delirium and stroke during the study period, as shown in figure 1. So, 52 participants [placebo group (n = 27) and probiotic group (n = 25)] finished the study.

Participants' characteristics are described in Table 1. The mean age in both groups was about 77 years. There is no significant difference in demographic features between the two groups, including age, sex, education, and marital status. The most common underlying diseases were hypertension, diabetes mellitus, dyslipidemia, and cardiovascular disease. Cardiometabolic factors, and BMI, were also not substantially different. Comparing nutritional assessment between the two groups, we found that total dietary intakes of fiber (p=0.01), calcium (p=0.03), and selenium (p=<0.01) were significantly different between subjects in both groups (Table 2).

After 12 weeks, neither participant's weight nor BMI changed. A similar trend was found in metabolic factors (including FBS, lipid profiles, and serum albumin level), as shown in Table 3. HDL-c serum concentrations dropped slightly more in the placebo group (-6.3±14.3 mg/dl) than in the probiotic group (-0.3±7.2 mg/dl). Serum levels of 25(OH) vitamin D increased in participants who consumed probiotic supplements (8.9±18.6 ng/dl), whereas it decreased in patients in the placebo group (-1.1±16.2 ng/dl).

However, we observed significant time effects between baseline and week 12 for MMSE score and serum levels of HDL-c. It means that among participants in the probiotic group, MMSE scores increased more than those in the placebo group (placebo group: 0.74±2.2, probiotic group: 1.8±1.9; P= <0.001) and HDL-c serum concentrations (placebo group: -6.3±14.3 mg/dl, probiotic group: -0.3±7.2 mg/dl; P=0.04) decreased in the placebo group more than to the probiotic group over the

time. In addition, there were significant time pretreatment interaction effects for the serum concentrations of LDL-c, total cholesterol, and 25(OH)D. Such that LDL-c (placebo group: -7.7±42.4 mg/dl, probiotic group:13.7±30.2 mg/dl; P= 0.04) and total cholesterol (placebo group: -12.3± 45.2 mg/dl, probiotic group: 11.4±37 mg/dl; P= 0.04) concentrations over the intervention period decreased in placebo group while increased in the probiotic group. However, after a 12-week intervention, serum levels of 25(OH)D (probiotic group: 8.9±18.6 ng/dl placebo group: -1.1±16.2 ng/dl; P= 0.04) increased in the participants who consumed probiotics supplements whereas decreased in the placebo group.

At baseline, the MMSE score in both groups was almost the same (16.6±5.4: in the probiotic group vs. 16.1±5.4: in the placebo group). However, we observed significant time effects between baseline and week 12 for the MMSE score. It means that among participants in the probiotic group, MMSE scores increased more than those in the placebo group (placebo group: 0.74±2.2 vs. probiotic group: 1.8±1.9), but the difference between the two groups of testing was not statistically significant (P=0.53). At the end of the study, the cognitive function of both groups deteriorated, and only one person who received probiotics experienced cognitive improvement, which was not statistically significant. There were no significant differences in TG, FBS, and Serum Albumin between the two groups at baseline at the end of the study. After adjusting for covariates such as gender, age, and BMI at the beginning of the study, as well as the mean energy intake, we found no significant differences in our results (Table 4).

As dementia progress, gradual loss of cognitive function has a tremendous negative impact on a patient's physical performance. At the beginning of the study, the mean Barthel score in the

intervention and control groups was 18.5 ± 1.9 and 18.1±2.6, respectively. After 12 weeks, functional loss was less significant in probiotic recipients (P=0.43), as given in Table 3.

Discussion

Dementia is the most prevalent and burdensome neurodegenerative disease, related to the remarkable morbidity and mortality rate in older adults(28, 29). As the world population is aging, finding an effective way to prevent and treat dementia is considered a global health challenge. Recently, probiotics as live microorganisms received significant attention due to their potential beneficial properties in cognitive abilities(30, 31). Probiotics might affect the central nervous system (CNS) through several direct and indirect pathways(32) and change CNS biochemistry by impacting brain-derived neurotrophic factors (BDNFs), serotonin, and gamma-aminobutyric acid (GABA), and dopamine. Therefore, its properties could modify human behavior and cognition (33-36). Likewise, probiotics can inhibit the release of inflammatory cytokines and diminish oxidative stress by increasing antioxidants, including superoxide dismutase and glutathione peroxidase (7, 37-39). Another potential advantage of probiotics is altering gut microbiota composition and elevating the diversity of beneficial bacteria. Probiotics increased short-chain fatty acid and tryptophan production, indirectly affecting CNS function(32).The present study aimed to evaluate the effect of probiotic supplementation on cognitive function, physical performance, metabolic factors, vitamin D, and albumin levels in older patients with Alzheimer's disease. Our findings demonstrated that supplementation with probiotics for 12-week had no considerable effect on these parameters before or after adjustment.

These findings confirm the results from prospective studies(22, 40) that no improvement in cognitive function of dementia patients was reported after taking probiotics. Our results for

physical performance with probiotics were in line with two other prospective studies (21, 41). In contrast to our results, in a study on 27 older adults, administered probiotic containing Bifidobacterium breve A1 for 24 weeks showed a considerable improvement in cognitive function and reduced the risk of dementia compared to the control group (42). In the same way, another clinical trial revealed such a beneficial effect (20). The small sample size, lack of a placebo group, and difference in strains and doses of administrated probiotics might explain these discrepancies. More studies need to shed further light on understanding the effect of supplementation with probiotics on cognitive behavior.

Another outcome of this study is that taking supplemental probiotics does not affect serum levels of vitamin D, albumin, FBS, and lipid profile. This finding is similar to the studies that did not report a significant effect of probiotics on these parameters (43-45). However, it was different from a clinical trial that showed that supplementation with probiotics for 12 weeks exerted a beneficial effect on some of the metabolic parameters including cholesterol, and triglyceride levels among 79 patients with Alzheimer's disease(46). In addition, Akbari et al. reported a remarkable effect on triglyceride after 200 ml/day of probiotic milk consumption for 12 weeks(25). An interventional study investigated a considerable rise in plasma albumin levels by taking for 12 weeks in patients with memory complaints (42). Moreover, another trial evaluating the effects of probiotic administration on 127 Canadian otherwise healthy hypercholesterolemia adults demonstrated a significant increase in serum vitamin D concentrations after nine weeks (47). The conflicting results of published clinical trials could be related to their methodology, including different intervention periods, analysis methods, and potential covariates.

Furthermore, using different doses and strains of probiotics may also have contributed to the inconsistent findings. There is no common consent about the most effective dose and strain of probiotics (48). This issue needs further exploration to be addressed.

Our study has several limitations. We could neither conduct stool analysis to evaluate the bacteria alteration in the gut nor assess the effect of probiotic administration on inflammatory cytokines due to resource limitations. Participation in this trial was decreased due to the COVID19 pandemic. Finally, probiotics supplement contains several strains of bacteria with different doses; thus, we could not find which strains had a favorable effect among dementia patients in the present clinical trial.

Conclusion

In conclusion, this clinical trial study revealed that supplementation with probiotics for 12-week had no favorable effect on cognitive function, ADLs, lipid profiles, vitamin D concentration, and serum albumin level in older patients with Alzheimer's disease. Further studies with larger sample size and a more extended follow-up period are needed to clarify the impact of probiotic supplementation on the elderly.

Data availability

Data supporting the conclusion of the study are available through contacting to the corresponding author.

Conflicts of interest

All authors have no conflict of interest.

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CRediT author statement

Mahdieh Mehmandoust: Conceptualization, Methodology, Writing - Original Draft. **Shima Raeesi**: Methodology, Writing- Reviewing and Editing. **Rezvan Hashemi**: Writing- Reviewing and Editing. **Mohammad Bidkhori**: Methodology, Software. **Alireza Namazi Shabestari**: Resources, Conceptualization, **Fatemeh Dashti**: Formal analysis. **Farzaneh Asoudeh**: Writing - Original Draft. **Zahra Vahabi**: Supervision

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Table 1. Baseline characteristics of the participants*

	Placebo group (n=27), mean± SD	**Probiotic group (n=25), mean ± SD**	**P-value[a]**
Age (y)	77.3 ± 8.4	77 ± 8.5	0.92
Height (cm)	158.1 ± 8.5	157.3 ± 14.9	0.81
Weight at baseline (kg)	66.6 ± 10.4	69.8 ± 10.1	0.26
Weight at end-of-trial (kg)	67.3 ± 10.4	69.8 ± 10.5	0.38
Weight change (kg)	0.3 ± 3.3	0.5 ± 3	0.81
BMI at baseline (kg/m2)	26.7 ± 4.6	28.7 ± 6.7	0.16
BMI at end-of-trial (kg/m2)	26.9 ± 4.7	28.8 ± 7	0.25
BMI change (kg/m2)	0.1 ± 1.3	0.2 ± 1.2	0.73
Sex (%female)	44.4	64	0.16[b]
Marital status (%married)	70.3	60	0.43[b]
Educational status (%diploma and higher)	7.4	8	0.44[b]
Current smoker (%)	11.1	12	0.92[b]
Diabetes (%)	18.5	32	0.26[b]
Hypertension (%)	55	52	0.53[b]
Dyslipidemia (%)	25.9	36	0.43[b]
Cardiovascular disease (%)	33.3	36	0.84[b]

*Data are means ± SD, [a] Obtained from independent t-test. [b] Pearson Chi-square test. BMI: body mass index.

Table 2. Dietary intakes of study participants throughout the study*

	Placebo group (n=27), mean ± SD	**Probiotic group (n=25), mean ± SD**	**P-value[a]**
Energy (kcal/day)	1895.07 ± 89.59	1853.16 ± 99.87	0.1
Carbohydrates (g/day)	243.73 ± 33.22	234.92 ± 40.5	0.3
Protein (g/day)	54.11 ± 8.16	52.75 ± 8.95	0.5
Fat (g/day)	81.46 ± 15.19	80.86 ± 11.26	0.8
Fiber (g/day)	15.22 ± 3.42	12.96 ± 3.09	0.01
SFAs (g/day)	16.95 ± 3.89	18.59 ± 5.18	0.2
MUFAs (g/day)	33.95 ± 7.98	34.37 ± 4.95	0.8
PUFAs (g/day)	24.34 ± 5.85	23 ± 4.82	0.3
Calcium (mg/day)	435.45 ± 123.43	534.84 ± 190.05	0.03
Selenium (µg/day)	0.0456 ± 0.0311	0.0278 ± 0.011	0.009
Zinc (mg/day)	6.99 ± 1.1	7.21 ± 1.75	0.4
Iron (mg/day)	10.64 ± 2.68	10.17 ± 2.64	0.5

*Data are means ± SD, [a] Obtained from independent t-test. SFA: saturated fatty acid, MUFAs: monounsaturated fatty acid; PUFAs: polyunsaturated fatty aci

Table 3. Means (±SEM) of MMSE, Barthel Index and metabolic status at study baseline and after 12 weeks in both placebo and intervention (probiotic) groups.

Characteristics	Placebo group (n=27)			Probiotic group (n=25)			P[1]		
	Baseline	End of trial	Change	Baseline	End of trial	Change	Time	Treatment	Time× treatment interaction
MMSE	16.1 (1.04)	16.8 (1.1)	0.74 (2.2)	16.6 (1.08)	18.4 (1.1)	1.8 (1.9)	<0.001	0.48	0.08
Barthel Index	18.07 (0.4)	17.7 (0.6)	-0.28 (1.8)	18.4 (0.4)	18.3 (0.6)	-0.16 (1.9)	0.37	0.51	0.79
FBS (mg/dl)	108.2 (5.7)	106.8 (6.2)	-1.5 (15.8)	108.1 (5.9)	113.02 (6.4)	4.9 (25.2)	0.58	0.71	0.27
HDL (mg/dl)	48.11 (2.5)	41.7 (1.5)	-6.3 (14.3)	42.2 (2.6)	41.9 (1.5)	-0.3 (7.2)	0.04	0.26	0.06
LDL (mg/dl)	109.5 (8.3)	101.7 (8.1)	-7.7 (42.4)	98.1 (8.6)	111.8 (8.4)	13.7 (30.2)	0.63	0.95	0.04
Total Cholesterol (mg/dl)	182.7 (9.8)	170.4 (8.7)	-12.3 (45.2)	169.8 (10.2)	181.2 (9.1)	11.4 (37)	0.88	0.93	0.047
TG (mg/dl)	125.2 (12.2)	121.3 (11.8)	-3.9 (6.6)	134.6 (12.7)	138.04 (12.3)	3.4 (58.2)	0.96	0.40	0.65
Alb (g/dl)	4.17 (0.06)	4.17 (0.1)	-0.01 (0.7)	4.06 (0.07)	4.11 (0.1)	0.5 (0.3)	0.75	0.40	0.73
25(OH)Vit D (ng/dl)	36.5 (2.8)	35.5 (2.9)	-1.1 (16.2)	29.9 (3.0)	38.8 (3.1)	8.9 (18.6)	0.14	0.63	0.04

[1] A 2-factor repeated-measures ANOVA was used to assess time effects and to analyze the time × treatment (probiotic or placebo) interaction effects on all dependent variables.

MMSE: mini-mental state examination, FBS: fasting blood glucose, HDL: high density lipoprotein, LDL: low density lipoprotein, TG: triglyceride, Alb: albumin, 25(OH)Vit D: 25-hydroxyvitamin D

Table 4. Adjusted Means (±SEM) of MMSE, Barthel Index and metabolic status at study baseline and after 12 weeks in both placebo and probiotic groups*.

characteristics	Placebo group (n=27)			Probiotic group (n=25)			P[2]		
	Baseline	End of trial	Change	Baseline	End of trial	Change	Time	Treatment	Time× treatment interaction
MMSE	16.1 (1.07)	16.8 (1.1)	0.7 (2.1)	16.6 (1.1)	18.4 (1.2)	1.7 (1.9)	0.11	0.53	0.051
Barthel Index	17.9 (0.4)	17.7 (0.6)	-0.3 (1.8)	18.6 (0.4)	18.3 (0.6)	-0.1 (1.8)	0.28	0.43	0.88
FBS (mg/dl)	108.7 (5.9)	108.5 (6.3)	-1.4 (15.8)	107.6 (6.2)	111.2 (6.6)	4.9 (25.2)	0.67	0.92	0.54
HDL (mg/dl)	47.6 (2.6)	41.05 (1.5)	-6.3 (14.3)	42.6 (2.7)	42.6 (1.6)	-0.3 (7.1)	0.13	0.54	0.05
LDL (mg/dl)	107.6 (8.08)	100.4 (7.7)	-7.7 (43)	100.23 (8.4)	113.33 (8.09)	13.6 (30.2)	0.10	0.79	0.06
Total Cholesterol (mg/dl)	179.8 (9.7)	168.4 (8.4)	-12.2 (45.9)	172.9 (10.1)	183.4 (8.8)	11.4 (37)	0.12	0.74	0.08
TG (mg/dl)	125.5 (12.9)	122.4 (12.3)	-3.8 (57.6)	134.3 (13.4)	136.8 (12.8)	3.3 (58.2)	0.54	0.48	0.75
Alb (g/dl)	4.17 (0.07)	4.2 (0.1)	-0.02 (0.7)	4.05 (0.07)	4.07 (0.1)	0.05 (0.3)	0.11	0.28	0.97
25(OH)Vit D (ng/dl)	36.4 (2.6)	35.4 (3.1)	-1.06 (16.1)	30.03 (2.7)	38.8 (3.2)	8.9 (18.6)	0.40	0.67	0.053

*All values adjusted for sex and baseline age, BMI as well as mean energy intake

[1] A 2-factor repeated-measures ANOVA was used to assess time effects and to analyze the time × treatment (probiotic or placebo) interaction effects on all dependent variables.

MMSE: mini-mental state examination, FBS: fasting blood glucose, HDL: high density lipoprotein, LDL: low density lipoprotein, TG: triglyceride, Alb: albumin, 25(OH)Vit D: 25-hydroxyvitamin D