How probiotic bacteria influence the motor and mental behaviors as well as immunological and oxidative biomarkers in multiple sclerosis? A double blind clinical trial

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ABSTRACT

Background and aims: This clinical trial was carried out to assess the effects of probiotic on mental and motor behaviors, metabolic profiles in patients with multiple sclerosis (MS).
Methods: Forty-eight patients with MS were treated by probiotics or placebo for four months to determine clinical symptoms, mental health, and metabolic profiles.
Results: Probiotic decreased expanded disability status scale (-0.52 ± 0.04 vs. $+0.16 \pm 0.07$, P < 0.001), beck depression inventory (-5.08 ± 0.71 vs. -2.62 ± 0.78, P = 0.026), general health questionnaire-28 (-6.7 ± 1.17 vs. -3.04 ± 1.13, P = 0.03) and depression anxiety and stress scale (-12.54 ± 1.81 vs. -3.33 ± 2.26, P = 0.003). Probiotic reduced malondialdehyde (P < 0.001) and 8-hydroxy-2′-deoxyguanosine (P < 0.001). Probiotic resulted in a significant reduction in IL-6 (P = 0.01) and high-sensitivity C-reactive protein (P = 0.03), and a significant increase in IL-10 (P < 0.001) and nitric oxide levels (P = 0.012).
Conclusion: Through modulation of intestinal flora, the probiotic bacteria may improve clinical symptoms by balancing the inflammatory and anti-inflammatory responses, and adjusting the oxidative biomarkers in the MS patients.
Clinical registration: http://www.irct.ir: IRCT2017082234497N2.

1. Introduction

Multiple sclerosis is an autoimmune and inflammatory demyelinating disease related to central nervous system (CNS) that is characterized by spinal cord syndrome, brainstem or cerebellar syndrome cognitive impairment and optic neuritis (Sand, 2015). The disease affects 2,500,000 people worldwide (Browne et al., 2014). The pathophysiology of MS is multifactorial. It is documented that inflammatory factors (Kouchaki et al., 2017), oxidative stress pathways (Morel, Bijak, Niwald, Miller, & Saluk, 2017) and insulin resistance (Oliveira et al., 2014) play a pivotal role in pathophysiology of MS; all correlated with increasing expanded disability status scale (EDSS) which is known as a main criteria for severity of MS (Kouchaki et al., 2017; Morel et al., 2017; Oliveira et al., 2014). Also, the prevalence of anxiety and depression is high in patients with MS (Boeschoten et al., 2016).

The gut microbiota consists of a population of bacteria that inhabits the gut accounting for 70% of microbes in the human body (Bäckhed, Ley, Sonnenburg, Peterson, & Gordon, 2005). Gut microorganisms live in symbiosis with the host and affect human nutrition, metabolism, physiology, and immune development and function (Rinaldi et al., 2018). It plays an important role in autoimmunity, and gut microbial dysbiosis is known to be correlated with pathogenesis of MS (Chen, et al., 2016). Importantly, specific microbiome signatures can be considered as biomarkers to predict the clinical outcome or disease-modifying treatment efficiency in MS (Calvo-Barreiro, Eixarch, Montalban, & Espejo, 2017). Since some species of bacteria may worsen or improve MS the gut microbiota of MS patients may be modified based on therapeutic benefit (Ezendam & van Loveren, 2008; van den Hoogen, Laman, & t Hart, 2017). It can be reached by using probiotics, prebiotics, or synbiotics. Probiotics, as nonpathogenic microorganisms, are able to interact with the gut microbiota and provide health benefits (Rinaldi et al., 2018) so that probiotic bacteria with immunoregulatory properties are potentially considered to become a new therapeutic treatment for autoimmune diseases (Atarashi et al., 2013). Different

species of *Lactobacilli* and *Bifidobacteria* has been a favorite of researchers to study their effects on CNS dysfunction in neurological disorders (Akbari et al., 2016; Tamtaji et al., 2017).

Our previous study on patients with MS demonstrated that a multispecies probiotic supplement positively affected some antioxidants and metabolic biomarkers as well as mental health parameters (Kouchaki et al., 2016). In the present work we aimed to assess if support of the gut microbiota by a probiotic formulation consisting different species of *Lactobacilli* and *Bifidobacteria* including *Bifidobacterium infantis*, *Bifidobacterium lactis, Lactobacillus reuteri, Lactobacillus casei, Lactobacillus plantarum* and *Lactobacillus fermentum* (each 2×10^9 CFU/d) influences clinical symptom, mental health, biomarkers of inflammation and oxidative stress in patients with MS.

2. Material and methods

2.1. Trial design

Our study was performed from September 2017 through January 2018. This intervention was designed as a 16-week randomized, double-blind, placebo-controlled trial.

2.2. Participants

The inclusion criteria were people with ages between 20 and 60, course of disease relapsing–remitting MS (RRMS), identified according to McDonald criteria and an expanded disability status scale (EDSS) score ≤ 4.5 referred to the Neurology Clinic of Shahid Beheshti Hospital in Kashan city, Iran. The exclusion criteria were primary progressive MS (PPMS), secondary progressive MS (SPMS), clinical relapse and glucocorticoid therapy during the past one month, pregnancy, women who were lactating within the prior six month, patients with bearing nephrolithiasis within the prior five years and consumers of probiotic or synbiotic during the past three month. With the permission of patients their documents were evaluated in our neurology clinic and the basic and clinical data were recorded.

2.3. Ethics statements

This clinical evaluation was performed according to the Declaration of Helsinki. Informed consent and signature were received from all subjects before starting the intervention. The ethical committee of Kashan University of Medical Sciences (KUMS) approved the research proposal. In addition, the proposal is registered in the Iranian website for registration of clinical trials (http://www.irct.ir: IRCT2017082234497N2).

2.4. Study design

At first, all patients were matched for disease severity based on relapses, EDSS, type of medications, gender, age and BMI. Participants were then randomly divided into two groups including the group receiving probiotic (n = 24) and the group receiving placebo (n = 24) for 16 weeks. Participants were asked not to alter their routine physical activity or usual dietary intakes during the study and were asked not to consume any supplements other than those provided to them by the investigators.

We recorded for three-day food and physical activities at beginning of study, after the third, sixth and ninth weeks of the intervention and, end of the study.

2.5. Sample size

To calculate sample size, we used the standard formula suggested for clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%). Based on a previous study (Kouchaki et al., 2016), we used 0.60 as SD and 0.54 as the difference in mean (d) of EDSS as key variable. Based on this, we needed 20 persons in each group. Assuming 4 dropouts in each group, the final sample size was determined to be 24 persons per group.

2.6. Randomization

Randomization and blinding was performed before the allocation of patients in the testing groups. This work was performed using computer-generated random numbers that was hidden from the patients and researchers until the end of the analysis.

2.7. Intervention

In the probiotic group, patients received probiotic capsules (produced by Zist Takhmir Company, Tehran, Iran) containing *Bifidobacterium infantis*, *Bifidobacterium lactis, Lactobacillus reuteri, Lactobacillus casei, Lactobacillus plantarum* and *Lactobacillus fermentum* (each 2×10^9 CFU/d). Multistrains and multispecies probiotics usually demonstrate enhanced beneficial effects, which could be related to a higher colonization rate, the synergistic combination of strain-specific properties or even a symbiotic effect between several microorganisms (Gilgun-Sherki et al., 2004).

The patients in the placebo group received capsules containing maltodextrine, the carrier of the probiotic bacteria. The probiotic and placebo capsules were resembled in color, shape, size, packaging, smell and taste. The treatment was lasted for 16 weeks.

2.8. Treatment adherence

The patients of both groups received placebo/probiotic capsules. The participants were requested to consume the placebo/probiotic capsule after dinner. The patients were asked to regress unused capsules at each visit. The returned capsules were counted to determine number of the capsules consumed.

2.9. Anthropometric measurements

Using a standard scale, weight and height of the patients were measured in an overnight fasting condition in the beginning and end of study. We calculated BMI of the patients as:

$$\text{Weight}\,(\text{kg})/\text{height}\,(\text{m}^2)$$

2.10. Assessment of outcomes

The primary outcomes were EDSS and inflammatory factors. The secondary outcomes were mental health parameters, insulin resistance and oxidative stress factors.

2.11. Clinical assessment

In the beginning and end of the study EDSS, relapses rate, disease duration and medications were evaluated objectively by a neurologist.

2.12. Assessment of mental health

Mental health was evaluated using beck depression inventory (BDI), general health questionnaire-28 (GHQ-28) and depression anxiety and stress scale (DASS) at the baseline and end of study (Beck, Ward, Mendelsohn, Mock, & Erbaugh, 1961; Crawford & Henry, 2004; Goldberg & Hillier, 1979).

2.13. Blood samples collection

Fasting blood samples were obtained at the beginning and end of

study in the early morning. The blood samples centrifuged at 1465g for 10 min at room temperature and the serum and plasma were stored at −80 °C. Then, biochemical tests were performed as soon as possible after sample preparation.

2.14. Assessment of inflammatory factors

For measurement of serum high-sensitivity C-reactive protein (hs-CRP) we used an ELISA kit (LDN, Nordhorn, Germany). The Giess method was used for determination of plasma nitric oxide (NO) (Tatsch et al., 2011). Other inflammatory and anti-inflammatory factors including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) concentrations were determined by ELISA kits (sigma-aldrich, USA).

2.15. Assessment of oxidative stress biomarkers

Malondialdehyde (MDA), glutathione (GSH) and total antioxidant capacity (TAC) of plasma were measured by the thiobarbituric acid reactive substance method (Janero, 1990), the method of Beutler and Gelbart (1985) and ferric reducing antioxidant power method (Benzie & Strain, 1996), respectively. Also, other oxidative stress biomarkers including 8-hydroxy-2′-deoxyguanosine (8-OHdG) and superoxide dismutase (SOD) were evaluated by ELISA kits (MyBioSource).

2.16. Assessment of insulin resistance

Fasting plasma glucose (FPG) was measured by enzymatic kits (Pars Azmun, Tehran, Iran). Homeostasis model of assessment-estimated insulin resistance (HOMA-IR), homeostatic model assessment for B-cell function (HOMA-B) and the quantitative insulin sensitivity check index (QUICKI) were calculated based on suggested formulas. To measure serum insulin we used a Monobind kit (California, USA) (Pisprasert, Ingram, Lopez-Davila, Munoz, & Garvey, 2013).

2.17. Statistical methods

For evaluating whether the variables are normally distributed, Kolmogrov-Smirnov test was applied to the data. Unpaired student *t*-test was used to detect differences in anthropometric measures, EDSS, parameters of mental health and metabolic indicators between the two groups. To compare categorical variables, we used Pearson Chi-square test. Adjustment for changes in baseline values of biochemical variables, age and BMI at baseline was performed by analysis of covariance (ANCOVA). The P-value of < 0.05 was considered statistically significant. All statistical analyses used SPSS version 18.

3. Results

All participants in the placebo and probiotic groups were completed the study (n = 24 for each group) (Fig. 1). The rate of compliance in the present study was high, such that more than 90% of the prescribed capsules were consumed in both groups. There was no side effect after probiotic consumption in the patients.

The basic and clinical characteristics of the patients are summarized in Table 1. The *t*-test analysis showed that mean disease duration (4.2 ± 0.42 vs. 5.2 ± 0.76, years; P = 0.26), age (34.79 ± 1.06 vs. 36.54 ± 1.44, years; P = 0.33), height (168.4 ± 1.68 vs. 166.7 ± 1.84, cm; P = 0.49), weight (−0.2 ± 0.4 vs. −0.8 ± 0.17, kg; P = 0.17) and BMI change (−0.1 ± 0.15 vs. −0.3 ± 0.05, kg/m²; P = 0.2) at the baseline and end of study were not statistically different in the probiotic compared to placebo group (Table 1). We found no significant change in the mean dietary macro- and micro-nutrient intakes between the two groups throughout the trial (Data not shown).

The *t*-test analysis showed that the probiotic treatment led to a significant decrease in EDSS score (−0.52 ± 0.04 vs. +0.16 ± 0.07,

P < 0.001) compared to the placebo group (Table 2). The probiotic supplement also significantly decreased mental health scores including BDI (−5.08 ± 0.71 vs. −2.62 ± 0.78, P = 0.026), GHQ (−6.7 ± 1.17 vs. −3.04 ± 1.13, P = 0.03) and DASS (−12.54 ± 1.81 vs. −3.33 ± 2.26, P = 0.003). A pronounced decrease was observed in the plasma concentration of MDA (−0.31 ± 0.75 vs. +0.15 ± 0.79, µmol/L; P < 0.001), 8-OHdG (−6.72 ± 2.03 vs. +3.15 ± 1.57, ng/mL P < 0.001) in the probiotic compared to placebo group. Additionally, the intervention resulted in a significant reduction in plasma levels of IL-6 (−0.2 ± 0.1 vs. 0.07 ± 0.08, pg/ml; P = 0.01) and hs-CRP (−0.61 ± 0.58 vs. +1.07 ± 0.5, µg/mL; P = 0.03), and a significant increase in IL-10 (+0.46 ± 0.16 vs. −0.3 ± 0.22, pg/ml; P < 0.001) and NO (+2.87 ± 1.16 vs. −1.64 ± 1.27, µmol/L; P = 0.012). A trend toward a greater decrease in serum insulin (−3.58 ± 0.55 vs. −1.35 ± 0.89, µIU/mL; P = 0.04) and HOMA-IR (−1 ± 0.15 vs. −0.33 ± 0.23, P = 0.02) was observed in the probiotic group compared with its placebo counterpart. The intervention not considerably influenced the other biochemical profiles including SOD, GSH, TAC, TNF-α, FPG and QUICKI (Table 2).

Adjustment for changes in the baseline values of biochemical variables, age and BMI at baseline was performed by ANCOVA. Our finding showed that BDI, GHQ, SOD and TAC are altered after this adjustment. So that, BDI and GHQ were significant in the probiotic group compared to the placebo one. After adjustment there was no significant difference in BDI (P = 0.05) and GHQ (P = 0.13) between the two groups. On the other hand, while the serum level of SOD and TAC was not significant between the two groups they showed a post-adjustment significant difference (SOD, P = 0.02; TAC, P = 0.04) (Table 3).

4. Discussion

This study evaluated the effects of a probiotic supplementation on disease severity, mental health, insulin resistance, inflammation and oxidative stress in patients with MS. We found that four months bacteriotherapy had favorable effects on EDSS, DASS, MDA, 8-OHdG, SOD, TAC, IL-6, IL-10, NO, hs-CRP, insulin and HOMA-IR. However, the intervention not sufficiently affected the other biochemical parameters.

Dietary factors and lifestyle may exacerbate or ameliorate MS symptoms by modulating the inflammatory statuses in relapsing-remitting MS and in primary-progressive MS (Von Geldern & Mowry, 2012). This is achieved by regulating both the metabolic and inflammatory pathways in the human cell and the composition of gut microbiota (Riccio et al., 2016). It is reported that the gut microbiota importantly affect the balance between inflammatory and anti-inflammatory factor in immune responses in MS (Lee, Menezes, Umesaki, & Mazmanian, 2011) and play an key role in depression (Lv et al., 2017). This proposes was proved by our finding where consumption of the probiotic supplementation resulted in a decreased EDSS, anxiety and depression scores. Ait-Belgnaoui et al. reported that administration of probiotics suppressed overactivity of hypothalamic-pituitaryadrenal (HPA) axis due to psychological stress in rats (Ait-Belgnaoui et al., 2014). Bercik et al. demonstrated that, by influencing activity of enteric neurons, probiotics have beneficial effects on gut chronic inflammation-induced anxiety (Bercik et al., 2011). In the previous study we demonstrated that administration of a probiotic formula for 12 weeks in MS subjects leads to decreased EDSS, anxiety and depression scores (Kouchaki et al., 2016).

We showed that the probiotic supplement decreased the levels of hs-CRP and IL-6, and increased concentration of NO and IL-10. Improvement of clinical scores by probiotic bacteria is contributed to alteration of immune responses by inhibition of inflammatory cytokine and increased anti-inflammatory cytokine and T regulatory (Abdurasulova et al., 2016; Salehipour et al., 2017). Oral treatment with the probiotic VSL#3 was associated with the induction of anti-inflammatory peripheral immune responses and that interruption of the
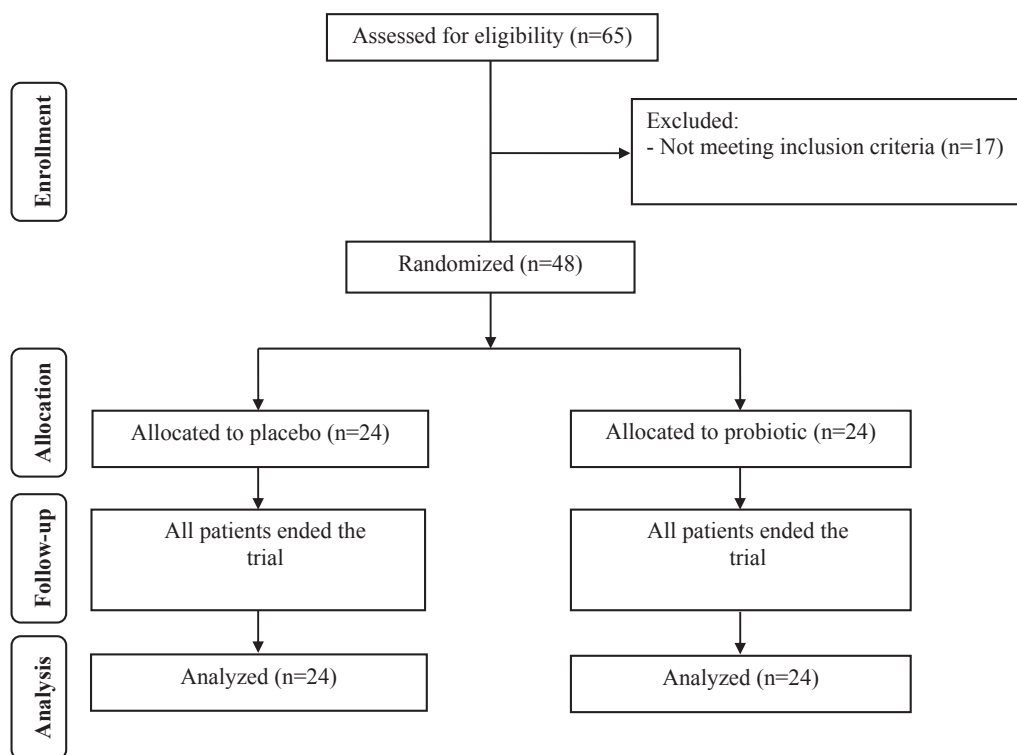
Fig. 1. Summary of patient flow.

Table 1
Basic and clinical characteristics of study participants.

		Placebo group (n = 24)	Probiotic group (n = 24)	P[a]
Number of subjects		24	24	–
Gender (%)	Male	6 (25)	6 (25)	> 0.99[b]
	Female	18 (75)	18 (75)	
Age (y)		36.54 ± 1.44	34.79 ± 1.06	0.33[a]
Disease duration (y)		5.2 ± 0.76	4.2 ± 0.42	0.26[a]
Height (cm)		166.7 ± 1.84	168.4 ± 1.68	0.49[a]
Weight (kg)	Study baseline	68.1 ± 1.94	70.16 ± 1.85	0.44[a]
	End of trial	67.2 ± 1.89	69.8 ± 1.82	0.31[a]
	Change	−0.8 ± 0.17	−0.2 ± 0.4	0.17[a]
BMI change (kg/m²)	Study baseline	24.5 ± 0.63	24.7 ± 0.55	0.81[a]
	End of trial	24.2 ± 0.62	24.6 ± 0.53	0.61[a]
	Change	−0.3 ± 0.05	−0.1 ± 0.15	0.2[a]
Interferon beta 1-α therapy (%)	Avonex	20 (83.3)	20 (83.3)	> 0.99[b]
	Rebif	4 (16.7)	4 (16.7)	

Data are presented as means ± SEM.
MS, multiple sclerosis; BMI, Body mass index.
[a] Obtained from independent *t* test.
[b] Obtained from Pearson Chi-square test.

patriotic treatment was associated with a decrease in IL-10-producing Treg cells (Tankou et al., 2018). In another study administration of *Lactobacillus* strains prevented and delayed the clinical signs in the EAE model of MS. In this study the probiotic supplement led to decreased levels of the pro-inflammatory cytokines (TNF-α, IFN-γ and IL-17) and increased levels of the anti-inflammatory cytokine IL-10 (Lavasani et al., 2010). Secher et al. found that probiotic administration decreased IL-6, increased IL-10 and improved clinical symptoms in an animal model of MS (Secher et al., 2017). Tankou et al. reported that administration of a probiotic supplement decreased frequencies of Th1 and Th17 in both healthy subjects and patients with MS (Tankou et al., 2018). Probiotics may improve MS associated symptoms through decreasing IL-6 and preventing infiltration of harmful T cells such as Th17 cells into the CNS (Yamashita et al., 2017).

We proved that taking the supplement led to significant reduction in insulin, insulin resistance, MDA and 8-OHdG levels, and increased SOD, TAC levels but did not affect plasma levels of GSH. In a study was shown that administration of probiotic for 12 weeks led to decreased MDA and HOMA-IR and increased GSH (Tamtaji et al., 2018). In addition, the significantly decreased HOMA-IR was seen among patients with non-alcoholic fatty liver disease after administration probiotic supplement for 12 weeks (Behrouz, Jazayeri, Aryaeian, Zahedi, & Hosseini, 2017). It has been reported that clinical symptoms and EDSS are correlated with increased oxidative stress and insulin resistance (Kouchaki et al., 2017; Morel et al., 2017; Oliveira et al., 2014). It is reported that 8-OHdG increased in patients with MS and that the change is correlated with clinical severity of disease and demyelinated brain lesion volume (Ljubisavljevic, Stojanovic, Basic, & Pavlovic, 2016). Suppressive effect of probiotic supplementations on some oxidative stressors in neurodegenerative disorders in clinical (Kouchaki et al., 2016) and experimental (Athari Nik Azm et al., 2018; Davari, Talaei, & Alaei, 2013) assessment has been confirmed.

Mechanistically, oxidative stress, NO and insulin resistance affects High-mobility group box 1 protein (HMGB1) releasing in inflammatory disease (Guzmán-Ruiz et al., 2014; Jiang & Pisetsky, 2006; Yu, Tang, & Kang, 2015). In turn, HMGB1 changes Treg/Th17 balance to Th17 through the up-regulation of TLR4-IL-6 pathway (Li et al., 2014). On the other hand, *Lactobacillus reuteri* and *Lactobacillus casei* induce regulatory T cells–produced IL-10 (Smits et al., 2005) which IL-10 suppresses IL-6 through the inhibition of nuclear factor-kappaB (NF-kappaB) activation (Heyen, Ye, Finck, & Johnson, 2000).

Taken together, it seems, through modulation of gut microbiota, the probiotic treatment may improve clinical symptoms by a balance in inflammatory and anti-inflammatory responses in MS patients. Further, decreased oxidative stressors might be involved in controlling the clinical symptoms in the patients with MS and EDSS (Carlson & Rose,

Table 2
Expanded disability status scale, parameters of mental health, biomarkers of inflammation and oxidative stress, and insulin at the study baseline and after 16 weeks intervention in patients with multiple sclerosis that received either probiotic supplement or placebo.

	Placebo group (n = 24)			Probiotic group (n = 24)			P[a]
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
EDSS	2.83 ± 0.2	3 ± 0.19	0.16 ± 0.07	2.85 ± 0.19	2.33 ± 0.17	−0.52 ± 0.04	< 0.001
BDI total scores	18.2 ± 1.52	15.58 ± 1.07	−2.62 ± 0.78	21.12 ± 1.35	16.04 ± 1.06	−5.08 ± 0.71	0.026
GHQ scores	28.5 ± 1.48	25.45 ± 1.5	−3.04 ± 1.13	33.16 ± 1.05	26.45 ± 1.39	−6.7 ± 1.17	0.03
DASS scores	41.29 ± 3.14	37.95 ± 4.02	−3.33 ± 2.26	51.62.0 ± 4.35	39.08 ± 3.93	−12.54 ± 1.81	0.003
SOD (U/mL)	51.61 ± 4.78	54.99 ± 5.42	3.37 ± 8.46	40.56 ± 3.22	48.48 ± 7.93	7.92 ± 8.55	0.7
8-OHdG (ng/ml)	9.92 ± 1.52	13.08 ± 1.21	3.15 ± 1.57	17.47 ± 1.44	10.74 ± 1.93	−6.72 ± 2.03	< 0.001
MDA (µmol/L)	2.81 ± 0.074	2.96 ± 0.072	0.15 ± 0.079	2.9 ± 0.06	2.59 ± 0.48	−0.31 ± 0.075	< 0.001
GSH (µmol/L)	506.57 ± 16.72	512.93 ± 13.91	6.36 ± 13.11	487.75 ± 13.47	529.4 ± 14.45	41.65 ± 16.84	0.1
TAC (mmol/L)	330.99 ± 8.13	315.51 ± 7.77	−15.48 ± 11.96	334.35 ± 11.9	336.4 ± 6.49	2.05 ± 9.81	0.26
IL-6 (pg/ml)	1.05 ± 0.07	1.13 ± 0.08	0.07 ± 0.08	1.4 ± 0.12	1.2 ± 0.11	−0.2 ± 0.1	0.01
IL-10 (pg/ml)	1.95 ± 0.23	1.65 ± 0.15	−0.3 ± 0.22	2.42 ± 0.2	2.88 ± 0.2	0.46 ± 0.16	< 0.001
TNF-α (pg/ml)	2.48 ± 0.19	2.7 ± 0.26	0.21 ± 0.3	3.02 ± 0.25	2.82 ± 0.22	−0.19 ± 0.11	0.21
hs-CRP (µg/mL)	2.66 ± 0.43	3.73 ± 0.48	1.07 ± 0.5	3.19 ± 0.64	2.58 ± 0.45	−0.61 ± 0.58	0.03
NO (µmol/L)	43.37 ± 0.86	41.72 ± 0.82	−1.64 ± 1.27	42.94 ± 0.98	45.82 ± 0.83	2.87 ± 1.16	0.012
FPG (mg/dL)	94.54 ± 2.69	91.12 ± 2.51	−3.41 ± 3.41	96.2 ± 4.13	87.04 ± 2.38	−9.16 ± 3.38	0.23
Insulin (µIU/mL)	10.94 ± 0.56	9.59 ± 1.05	−1.35 ± 0.89	11.84 ± 0.79	8.25 ± 0.87	−3.58 ± 0.55	0.04
HOMA-IR	2.55 ± 0.14	2.22 ± 0.27	−0.33 ± 0.23	2.81 ± 0.2	1.81 ± 0.21	−1 ± 0.15	0.02
QUICKI	0.33 ± 0.002	0.35 ± 0.006	0.01 ± 0.005	0.33 ± 0.004	0.36 ± 0.008	0.3 ± 0.006	0.11

Data are means ± SEM.
BDI, Beck depression inventory; DASS, Depression anxiety and stress scale; EDSS, Expanded disability status scale; FPG, Fasting plasma glucose; GHQ, General health questionnaire; GSH, Total glutathione; HOMA-IR, Homeostasis model of assessment-estimated insulin resistance; hs-CRP, High-sensitivity C-reactive protein; IL-6, Interleukins-6; IL-10, Interleukins-10; MDA, Malondialdehyde; NO, Nitric oxide; QUICKI, Quantitative insulin sensitivity check index; SOD, Superoxide dismutase; TAC, Total antioxidant capacity; TNF-α, Tumor necrosis factor-α; 8-OHdG, 8-hydroxy-2′ -deoxyguanosine.

[a] P values represent the time × group interaction (computed by analysis of the t test).

Table 3
Adjusted changes in expanded disability status scale and biochemical parameters in patients with multiple sclerosis that received either probiotic or placebo.

	Placebo group (n = 24)	Probiotic group (n = 24)	P[a]
EDSS	0.15 ± 0.05	−0.51 ± 0.05	< 0.001
SOD (U/mL)	12.22 ± 4.03	0.92 ± 4.03	0.02
8-OHdG (ng/mL)	0.78 ± 1.66	−4.35 ± 1.66	0.04
MDA (µmol/L)	0.11 ± 0.5	−0.27 ± 0.5	< 0.001
GSH (µmol/L)	12.05 ± 12.39	35.96 ± 12.39	0.18
TAC (mmol/L)	−17.46 ± 7.15	4.03 ± 7.15	0.04
IL-6 (pg/ml)	0.91 ± 0.09	−0.21 ± 0.09	0.03
IL-10 (pg/ml)	−0.41 ± 0.16	0.56 ± 0.16	< 0.001
TNF-α (pg/ml)	0.12 ± 0.22	−0.09 ± 0.22	0.51
hs-CRP (µg/mL)	0.98 ± 0.42	−0.52 ± 0.42	0.01
NO (µmol/L)	−1.52 ± 0.83	2.75 ± 0.83	0.001
Insulin (µIU/mL)	−1.34 ± 0.76	−3.59 ± 0.76	0.04
HOMA-IR	−0.33 ± 0.2	−0.99 ± 0.2	0.02
QUICKI	0.017 ± 0.006	0.032 ± 0.006	0.9
GHQ	−3.57 ± 1.17	−6.17 ± 1.17	0.13
DASS	−3.44 ± 2.05	−12.43 ± 2.05	0.004
BDI	−3.06 ± 0.54	−4.64 ± 0.54	0.05

All values are means ± SEM.
BDI, Beck depression inventory; DASS, Depression anxiety and stress scale; EDSS, Expanded disability status scale; FPG, Fasting plasma glucose; GHQ, General health questionnaire; GSH, Total glutathione; HOMA-IR, Homeostasis model of assessment-estimated insulin resistance; hs-CRP, High-sensitivity C-reactive protein; IL-6, Interleukins-6; IL-10, Interleukins-10; MDA, Malondialdehyde; NO, Nitric oxide; QUICKI, Quantitative insulin sensitivity check index; SOD, Superoxide dismutase; TAC, Total antioxidant capacity; TNF-α, Tumor necrosis factor-α; 8-OHdG, 8-hydroxy-2′ -deoxyguanosine.

[a] Obtained from analysis of ANCOVA adjusted for baseline values + age and baseline BMI.

2006; Gilgun-Sherki, Melamed, & Offen, 2004). Emerging evidence highlights that the gut microbiota modification open a new window for treatment of the autoimmune disease MS (Secher et al., 2017; Tankou et al., 2018). However, more preclinical and clinical studies are required to signature the therapeutic potentials of probiotic bacteria in the treatment of patients with MS. The limitation of the study was difficulty in counting microbial flora in the MS patients. Future prospects in MS research should regard effects of probiotic supplement on gut microbiota composition, NF-kB, HMGB1 and T and B cells in patients with MS.

5. Conclusion

Overall, the administration of probiotic bacteria may influence the motor and mental behaviors by modulation of inflammatory and oxidative biomarkers in patients with MS. The probiotic supplements could be a new strategy for improving and controlling MS severity.

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Conflicts of interest

No conflicts of interest.

Author contributions

MS contributed in conception, data collection and manuscript drafting. O-RT, EK and ZA contributed in conception, data collection and manuscript drafting. All authors read and approved the final version of the paper.

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