












Research Article

The Effects of Probiotic Supplementation on Opioid-Related Disorder in Patients under Methadone Maintenance Treatment Programs

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Received 6 October 2021; Accepted 11 March 2022; Published 30 March 2022

Academic Editor: Andrea Scribante

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Introduction. Patients under methadone maintenance treatment programs (MMTPs) are susceptible to numerous complications (e.g., mental and metabolic disorders). This study evaluated the effects of probiotics on clinical symptoms, biomarkers of oxidative stress, inflammation, insulin resistance, and serum lipid content in patients receiving MMTPs. **Materials and Methods.** A randomized, double-blind, placebo-controlled trial was conducted among 70 patients receiving MMTPs to receive either 1.8×10^9 CFU/day probiotics ($n = 35$) or placebo ($n = 35$) for 12 weeks. Clinical symptoms and metabolic profiles were measured before and after the intervention in patients receiving MMTPs. **Results.** Compared with the placebo group, probiotic supplementation resulted in a significant improvement in the severity of depression ($P < 0.05$). In addition, probiotic administration significantly decreased fasting plasma glucose (FPG), total cholesterol, and low-density lipoprotein cholesterol (LDL cholesterol) ($P < 0.05$). Furthermore, probiotics resulted in a significant reduction in high-sensitivity C-reactive protein (hs-CRP) and a significant elevation in total antioxidant capacity (TAC) and total glutathione (GSH) levels ($P < 0.05$). **Conclusion.** Treatment with probiotics for 12 weeks to patients receiving MMTPs had beneficial effects on symptoms of depression, as well as several metabolic profiles. Clinical Trial Registration: this study was registered in the Iranian website (<https://www.irct.ir>) for clinical trials registration (<https://fa.irct.ir/trial/46363/IRCT20170420033551N9>). The registration date is March 22, 2020.

1. Introduction

According to the United Nations [1], more than 180 million people around the world are suffering from drug addiction. One of the most worrying post-addiction problems is

malnutrition in all walks of life, whether in industrial or nonindustrial human societies [2]. In addition, overall body mass has been shown to increase predominantly due to an increase in the proportion of body fat upon methadone treatment [3]. A national pilot study was conducted in Iran

in 2002 to assess the feasibility of reducing drug-related adverse effects, leading to the establishment of private and public centers for methadone treatment [4]. Several reasons support the efficacy of methadone as an effective treatment for opioid use disorder, including cost-effectiveness and strong potential for managing the physical and psychological condition of opioid-like addiction [5]. Approximately 5,000 outpatient methadone maintenance treatment programs (MMTPs) and buprenorphine exist in Iran to treat opioid dependency, covering nearly half a million subjects [6]. It should be noted that there remain multiple unresolved challenges and questions despite many practical MMTPs. Following the implementation of MMTPs, opioid abusers experienced several metabolic (such as inflammation and oxidative stress) and mental (such as sleep, depression, and anxiety) disorders, which affect their quality of life and increase the frequent drug addiction [7, 8]. Treatment with methadone, compared with buprenorphine, was associated with increased frequency of metabolic syndrome. Both drugs are associated with obesity, overweight, and insulin resistance. Extended methadone treatment may cause altered triglycerides, blood pressure, HDL, fasting glucose, and hemoglobin A1C profiles [9]. In addition, previous reports on gut microbiota have explored the effects of drug use on bacterial composition and diversity. Patients under MMTPs altered gut microbiota compared with former and current drug users, likely due to effects of both dietary changes and direct intake of the opiate methadone, leading to enrichment for bacteria (e.g., *Bifidobacterium*, *Fusicatenibacter*, and *Lactobacillus*) and enhanced orexin-A [10].

Gut microbiota represents a diverse number of microorganisms in the digestive tracts, which influence host physiology, including the immune system and the central nervous system [11]. In addition, the gut microbiota is known to be related to opioid use, psychiatric disorders, and type 2 diabetes [12]. The gut microbiota can regulate cerebral performance and behavior by modulating several neuro-metabolic and neurochemical processes [13, 14]. Special attention has recently been paid to probiotics due to wide clinical purposes and health-promoting effects to manage several clinical conditions, such as chronic and acute gastrointestinal and non-gastrointestinal problems [15]. Probiotics have numerous health-promoting effects, and the gut microbiota are vital for the regulation of metabolic and mood processes [16]. Patients with multiple sclerosis showed an improvement in general health, depression, and anxiety and stress scales following twelve weeks of probiotic supplementation in a study by Kouchaki et al. [17]. The results of a meta-analysis in randomized control trials indicated a reduction in depression and anxiety levels in patients supplemented with probiotics [18]. Nevertheless, there are reports on the lack of effect of probiotics on mental health outcomes in obese pregnant women [19]. A systematic review and meta-analysis showed that malondialdehyde level, C-reactive protein, interleukin-10, and Hamilton Depression Scale were positively improved in psychiatric patients supplemented with probiotics [20]. Further, psychiatric patients treated with probiotics exhibited improved levels of HDL cholesterol, VLDL, triglycerides, insulin resistance,

insulin, malondialdehyde, and C-reactive protein. [21]. Beneficial activities have been reported for various probiotics, including enhanced absorption of nutrients, overexpression of brain-derived neurotrophic factor (BDNF), decreased levels of pro-inflammatory cytokines, free radical scavenging potential, overproduction of gamma-aminobutyric acid (GABA), regulation of critical neurotransmitters, and antioxidant activities. These activities may play a pivotal role in the pathology and physiology of metabolic and mental health processes [22–26].

A literature review revealed limited information on the effects of probiotic administration on depression, anxiety, sleep quality, lipid profiles, insulin sensitivity, inflammatory factors, and oxidative stress biomarkers in opioid-related disorder. Hence, the present research aimed to evaluate the influential activities of probiotics on clinical symptoms, biomarkers of oxidative stress, inflammation, insulin resistance, and serum lipid content among MMTP patients.

2. Materials and Methods

2.1. Participants. The study was conducted at the substance abuse Soltan Mirahmad Clinic, Kashan, Iran, between April 2020 and July 2020. This randomized, double-blind, placebo-controlled trial was registered in the Iranian Clinical Trials Registration of clinical trials (<https://fa.irct.ir/trial/46363/IRCT20170420033551N9>). The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences (approval code: IR.KAUMS.MEDN-T.REC.1398.116) and was conducted according to the Declaration of Helsinki. Informed consent was obtained from all patients prior to the enrollment. All informed consent forms were reviewed by the Research Ethics Committee of KAUMS.

2.2. Inclusion/Exclusion Criteria

2.2.1. Inclusion Criteria

- (1) Patients receiving MMTPs.
- (2) Aged 18 to 60 years.
- (3) Opioid use disorder as diagnosed by DSM-IV criteria.

2.2.2. Exclusion Criteria

- (1) Unwillingness to cooperate.
- (2) Taking probiotic, multivitamin-mineral, and antioxidant supplements during the last 3 months before the intervention.
- (3) Positive urine tests indicating the use of any substance or methamphetamine during intervention.
- (4) Current severe depression and mania, and psychosis as determined by a study physician.
- (5) Metabolic diseases.
- (6) Cardiovascular disorder.

2.3. Study Design. In this randomized, double-blind, placebo-controlled trial, after balanced block randomization, patients were assigned to receive either probiotic ($n = 35$) or placebo ($n = 35$) for 12 weeks during receiving MMTPs. Probiotics contained four viable and freeze-dried strains: *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium lactis* (1.8×10^9 CFU/g each; capsule). Probiotic supplements and placebos were produced by the LactoCare®, Takgene Pharma Company (Tehran, Iran). Random numbers were generated with computer software (Stat Trek) by trained staff at the Soltan Mirahmad Clinic. Randomization and allocation were concealed from the researchers and patients until the final analyses were completed. A person at the substance abuse treatment clinic, who was not involved in the trial and was unaware of random sequences, assigned the participants to the numbered bottles of supplements. At the clinic, patients received a weekly dose of methadone in the form of syrup, about half of the daily dose in the clinic and the remainder at home.

2.4. Assessment of Outcomes. Mental health is an important indicator of the health status of patients under MMTPs. Therefore, mental health (depression, anxiety, and sleep quality) was considered as the primary outcome, and lipid profiles, insulin metabolism, inflammatory factors, and oxidative stress biomarkers were considered as secondary outcomes.

2.5. Clinical Signs. In this study, Beck's Depression Inventory (BDI), Beck's Anxiety Inventory (BAI), and Pittsburgh Sleep Quality Index (PSQI) were used to assess the levels of depression, anxiety, and sleep quality, respectively [27–29]. BDI is a 21-question and BAI is a 20-question inventory in which each question was scored between 0 and 4; higher scores indicate higher levels of depression and anxiety. The Persian versions of both inventories were validated in the previous studies [30, 31]. PSQI is an applicable instrument used to measure the pattern and quality of sleep. It differentiates poor from good sleep quality by measuring seven components of sleep (e.g., subjective sleep quality, sleep duration, sleep latency, habitual sleep efficiency, use of sleeping medications, sleep disturbances, and daytime dysfunction) over the last month [29].

2.6. Biochemical Assessment. At baseline and after the 12-week intervention, fasting blood (10 mL) was collected from each patient at the substance abuse treatment center (Soltan Mirahmad Clinic). Serum insulin levels were measured with an ELISA kit (DiaMetra, Milano, Italy) with intra- and inter-assay CVs below 5%. The homeostasis model of assessment, QUICKI, and HOMA-IR were assessed using the established formulas [32]. Enzymatic kits (Pars Azmun, Tehran, Iran) with inter- and intra-assay CVs of less than 5% were used to measure fasting plasma glucose and lipid parameters. Serum hs-CRP levels were determined by the commercial ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs

below 7%. In addition, NO levels were assessed using the Griess method [33]. In addition, TAC levels were assessed using the ferric-reducing antioxidant power method developed by Benzie and Strain [34]. In addition, GSH and MDA levels were evaluated using Beutler et al.'s method and thiobarbituric acid reactive substance spectrophotometric test, respectively [35, 36]. CVs for plasma MDA, GSH, and TAC were less than 5%.

2.7. Statistical Analysis. The normality of data was assessed by the Shapiro–Wilk test using the Statistical Package for Social Science Version 22 (SPSS Inc., Chicago, Illinois). To detect the differences in anthropometric parameters between treatment groups, we used the independent-samples *t*-test and chi-square test. Multiple linear regression models were used to assess treatment effects on study outcomes (clinical sign parameters, lipid profiles, insulin sensitivity, inflammatory factors, and oxidative stress biomarkers) after adjusting for baseline levels of variables. The effect sizes were presented as the mean differences with 95% confidence intervals. *P* values < 0.05 were considered statistically significant.

3. Results

Of 101 screened patients with MMTPs, 70 patients were enrolled in the study and randomly assigned to either the intervention or control group to receive probiotics or placebo, respectively (35 people to each group). In the placebo group, four patients discontinued intervention due to positive methamphetamine urine test and personal reasons. In the intervention group, seven patients revoked their consents. Therefore, 59 patients (intervention ($n = 28$) and placebo ($n = 31$)) were analyzed. CONSORT flow diagram on the enrollment of patients in this study is shown in Figure 1. No side effects were ascertained following the administration of probiotics and placebos in patients receiving MMTPs. Patients' characteristics were analogous between the two groups (Table 1).

3.1. Clinical Signs. Compared with the placebo group, probiotics significantly decreased the level of depression (β 1.94; 95% CI, 0.14, 3.75; $P = 0.03$), respectively. In addition, no significant differences were indicated between the two groups in terms of the Beck's Anxiety Inventory and Pittsburgh Sleep Quality Index (Table 2).

3.2. Metabolic Profiles. After the 3-month intervention, probiotic supplementation significantly decreased FPG (β 8.53 mg/dL; 95% CI, 3.32, 13.75; $P = 0.002$), total cholesterol (β 15.14 mg/dL; 95% CI, 4.69, 25.60; $P = 0.005$), and LDL cholesterol (β 14.51 mg/dL; 95% CI, 2.29, 26.74; $P = 0.02$), compared with the placebo group. In addition, probiotic resulted in a significant reduction in hs-CRP (β 2.05 mg/L; 95% CI, 0.44, 3.66; $P = 0.01$) and significant enhancement of TAC (β -110.02 mmol/L; 95% CI, -189.33, -30.71; $P = 0.007$) and plasma GSH levels (β -66.33 μ mol/L; 95%

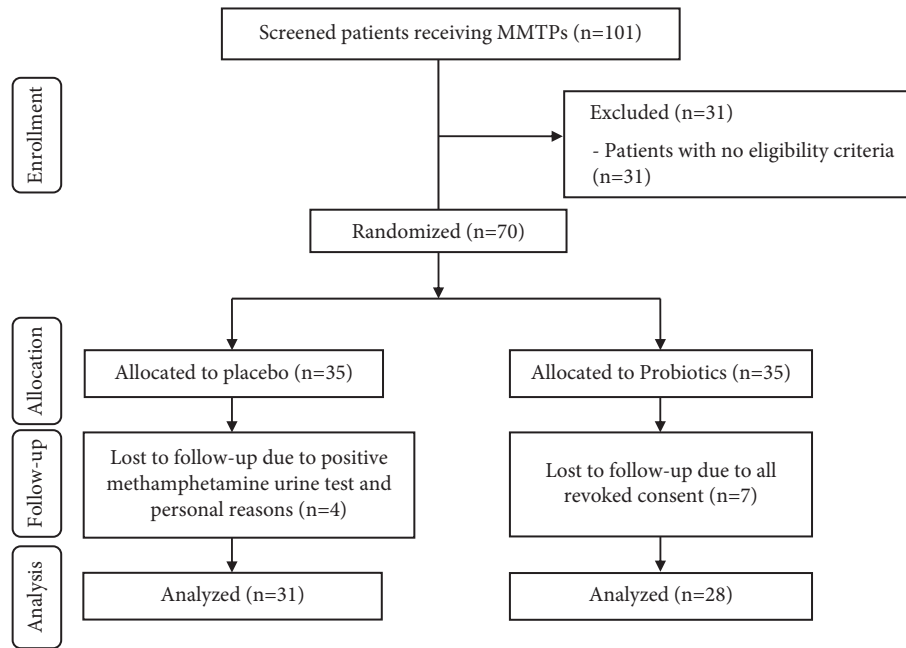


FIGURE 1: Summary of the patient flow diagram.

CI, -107.13 , -25.52 ; $P = 0.002$) compared with the placebo. There was no significant effect of probiotic supplementation on insulin metabolism, HOMA-IR, QUICKI, triglycerides, VLDL and HDL cholesterol, malondialdehyde levels, and plasma nitric oxide (Table 3).

4. Discussion

Our study investigated the effects of probiotics on clinical symptoms, lipid profiles, insulin sensitivity, inflammatory factors, and oxidative stress biomarkers in patients receiving MMTPs. The results showed that a 12-week probiotic supplementation in patients receiving MMTPs had beneficial effects on depression, glycemic control, total and LDL cholesterol, hs-CRP serum level, total antioxidants, and plasma glutathione, no effect on anxiety, sleep quality, insulin levels, HOMA-IR, QUICKI, triglycerides, VLDL and HDL cholesterol, malondialdehyde levels, and plasma nitric oxide. Addiction can cause mental health disorder, metabolic disorder, and oral complications and may result in relapse to drug abuse and reduced addiction treatment success rate [7, 8, 37]. There seems to be an increasing volume of evidence of an association between metabolic disorder and periodontitis [38]. The periodontal disease represents a progressive destruction of tooth-supporting tissues. Recently, paraprobiotics are regarded as an adjunctive therapy to the nonsurgical scaling and root planning [39]. On the other hand, gut homeostasis plays an important role in human and animal health. The disruption of gut homeostasis enhances the risk of infections and represents potential side effect of opioid use. The effects of opioids in the gut homeostasis, both chronic and acute, include persistent constipation and might worsen pain, mental health signs, and metabolic syndrome [40–42]. In addition, the gut microbiota in rats treated with methamphetamine demonstrated that Bacillaceae and

Ruminococcaceae were more abundant in the rats with methamphetamine-induced conditioned place preference [43]. So, gut microbial diversity might be shaped by addiction-associated behaviors and these can function as biological indicators to evaluate the health of people with a history of drug use. Also, disturbances in mental health parameters and metabolic profiles have previously been documented in opioid users and patients receiving MMTPs [7, 44]. This evidence suggested a potential link involving the gut microbiota, metabolism, and psychological factors that warrant consideration therapeutically. This is the first study to investigate the effects of probiotics on clinical symptoms, lipid profiles, insulin sensitivity, inflammatory factors, and biomarkers of oxidative stress in patients receiving MMTPs.

4.1. The Effect of Probiotics on Clinical Symptoms. The prevalence of mental disorders in patients receiving MMTPs is ten-fold higher than in the general population and two- to three-fold higher than those with substance abuse. In addition, the high prevalence of sleep disorders in patients receiving MMTPs has been estimated at 84% [45, 46]. A growing body of evidence has linked mental health symptoms to the gut microbiome, demonstrating that the latter might be modulating the gut-brain axis [47]. Disturbances in the microbiome have been implicated in both mental and physical illnesses. Signs of psychiatric disturbance, such as depression, anxiety, and autism, have been posited to be associated with disturbed gut microbiota [47, 48]. Opioid use significantly affects patients' diets and leads to malnutrition. Numerous studies have reported the effects of drug abuse on nutrition and nutritional deficiencies, and a beneficial effect of probiotics as a food or aromatic plant product in nutrition and diet has been advanced [49–51]. The gut microbiome as a therapeutic target for mental health

TABLE 1: Characteristics of patients receiving MMTPs¹.

	Placebo (<i>n</i> = 31)	Probiotics (<i>n</i> = 28)	<i>P</i> ²
Age (year)	47.6 ± 8.4	44.4 ± 10.3	0.20
Age first experience of drug use (%)	20.6 ± 7.5	21.0 ± 7.6	0.83
<i>The first type of consumable (%)</i>			
Opium and opium ashes	15 (48.4)	16 (57.1)	0.34 [†]
Heroin or cracked heroin	8 (25.8)	3 (10.7)	
Marijuana	6 (19.4)	6 (21.4)	
Methamphetamine	2 (6.5)	1 (3.6)	
Other different substances	0 (0)	2 (7.1)	
<i>Psychotropic medications (%)</i>			
Sedative hypnotics	2 (6.5)	4 (14.3)	0.59 [†]
Antidepressants	3 (9.7)	2 (7.1)	
None	26 (83.9)	22 (78.6)	
Height (cm)	171.6 ± 15.4	173.9 ± 8.6	0.49
Weight at study baseline (kg)	72.5 ± 13.9	83.0 ± 23.5	0.04
Weight at the end of trial (kg)	72.6 ± 13.9	82.2 ± 22.4	0.05
BMI at study baseline (kg/m ²)	25.1 ± 6.6	27.3 ± 6.6	0.21
BMI at the end of trial (kg/m ²)	25.3 ± 7.7	27.0 ± 6.3	0.35
<i>Education (%)</i>			
Illiterate	2 (6.5)	3 (10.7)	0.34 [†]
Elementary	13 (41.9)	13 (46.4)	
Intermediate	15 (48.4)	8 (28.6)	
Diploma	0 (0)	2 (7.1)	
High educated	1 (3.2)	2 (7.1)	
<i>Marital status (%)</i>			
Single	8 (25.8)	10 (35.7)	0.37 [†]
Married	19 (61.3)	17 (60.7)	
Widow/divorced	4 (12.9)	1 (3.6)	
<i>Job (%)</i>			
Unemployed	10 (32.3)	8 (28.6)	0.91 [†]
Employed	5 (6.1)	4 (14.3)	
Others	16 (51.6)	16 (57.1)	
MMT dose (mL/d)	19.5 ± 13.3	19.5 ± 6.6	0.98
In-person daily dosing (mL)	9.8 ± 6.7	9.9 ± 3.2	0.94
Take-home dosing (mL/weekly)	127.2 ± 86.7	126.8 ± 43.1	0.98
Duration of MMT (y)	7.5 ± 4.3	9.8 ± 5.3	0.08

¹Data are mean ± SDs and percentage. ²Obtained from independent *t*-test. [†]Obtained from the Pearson chi-square test.

TABLE 2: Means (±standard deviation) of clinical sign parameters at baseline and after the 12-week intervention in patients receiving MMTPs¹.

Variables	Placebo (<i>n</i> = 31)		Probiotics (<i>n</i> = 28)		Difference in outcome measures between probiotics and placebo treatment groups ¹	
	Baseline	Week 12	Baseline	Week 12	β (95% CI)	<i>P</i> ²
BDI	20.5 ± 10.4	19.5 ± 10.3	25.2 ± 14.3	22.1 ± 13.2	1.94 (0.14, 3.75)	0.03
BAI	13.7 ± 10.3	12.2 ± 8.9	14.8 ± 10.0	11.2 ± 8.2	1.69 (−0.08, 3.48)	0.06
PSQI	4.0 ± 1.7	4.0 ± 1.6	5.3 ± 2.2	4.8 ± 1.9	0.007 (−0.58, 0.60)	0.98

Data are mean ± SDs. ¹“Outcome measures” refers to the change in values of measures of interest between baseline and week 12. β (difference in the mean outcome measures between treatment groups (probiotic group = 1 and placebo group = 0)). ²Obtained from multiple regression models. BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PSQI, Pittsburgh Sleep Quality Index.

disorder has been a focus in psychiatric research, among many others [52]. In our study, a 12-week probiotic supplementation in patients receiving MMTPs reduced depression scores but had no effect on anxiety scores and sleep quality. It has previously been reported that the use of probiotics has a beneficial effect on mental health and cognitive function [53]. Recent studies in animals and humans have shown that interventions involving intestinal microbiota, including probiotics, may be an effective

treatment for mental health disorders [54, 55]. In an animal study, Li et al. reported that probiotics may reduce anxiety and depression [56]. Furthermore, young healthy women who received probiotics containing two strains of *Lactobacillus helveticus* and *Bifidobacterium longum* for 30 days experienced significant improvements in their mental health [57]. It has also been reported that long-term probiotic supplementation in rugby players may have beneficial effects on their sleep quality and muscle soreness during training

TABLE 3: Means (\pm standard deviation) of lipid profiles, insulin sensitivity, inflammatory factors, and oxidative stress biomarkers at baseline and after the 12-week intervention in patients receiving MMTPs¹.

Variables	Placebo ($n = 31$)		Probiotics ($n = 28$)		Difference in outcome measures between probiotics and placebo treatment groups ¹	
	Baseline	Week 12	Baseline	Week 12	β (95% CI)	P^2
FPG (mg/dL)	90.3 \pm 18.9	89.1 \pm 21.0	86.4 \pm 15.1	77.3 \pm 11.5	8.53 (3.32, 13.75)	0.002
Insulin (μ U/mL)	13.0 \pm 4.4	11.7 \pm 5.5	14.3 \pm 5.0	10.3 \pm 5.4	1.94 (−0.51, 4.40)	0.11
HOMA-IR	3.0 \pm 1.1	2.6 \pm 1.2	3.1 \pm 1.5	2.1 \pm 1.1	0.43 (−0.08, 0.96)	0.09
QUICKI	0.33 \pm 0.02	0.34 \pm 0.03	0.32 \pm 0.02	0.34 \pm 0.07	0.001 (−0.02, 0.02)	0.95
Triglycerides (mg/dL)	142.5 \pm 84.4	141.7 \pm 86.0	151.4 \pm 65.3	142.0 \pm 64.6	7.51 (−3.30, 18.33)	0.16
VLDL cholesterol (mg/dL)	28.5 \pm 16.8	28.3 \pm 17.2	30.2 \pm 13.0	28.4 \pm 12.9	1.50 (−0.66, 3.66)	0.16
Total cholesterol (mg/dL)	174.1 \pm 47.1	172.4 \pm 46.6	187.6 \pm 57.5	168.5 \pm 49.5	15.14 (4.69, 25.60)	0.005
LDL cholesterol (mg/dL)	101.6 \pm 54.8	99.4 \pm 52.4	116.0 \pm 56.2	97.5 \pm 48.7	14.51 (2.29, 26.74)	0.02
HDL cholesterol (mg/dL)	43.9 \pm 10.4	44.6 \pm 9.1	41.3 \pm 10.1	42.5 \pm 8.9	−0.51 (−3.82, 2.78)	0.75
Hs-CRP (mg/L)	6.2 \pm 3.0	7.3 \pm 3.9	6.0 \pm 4.1	5.0 \pm 4.1	2.05 (0.44, 3.66)	0.01
Total nitrite (μ mol/L)	50.0 \pm 13.3	52.3 \pm 13.6	52.4 \pm 13.9	54.5 \pm 14.7	0.48 (−2.47, 3.44)	0.74
TAC (mmol/L)	928.3 \pm 174.5	941.5 \pm 185.4	886.1 \pm 184.2	1016.9 \pm 143.1	−110.02 (−189.33, −30.71)	0.007
GSH (μ mol/L)	655.8 \pm 168.4	691.6 \pm 161.3	626.9 \pm 149.8	728.4 \pm 148.0	−66.33 (−107.13, −25.52)	0.002
MDA (μ mol/L)	2.8 \pm 0.5	3.2 \pm 0.4	2.7 \pm 0.4	3.3 \pm 0.4	−0.14 (−0.37, 0.08)	0.20

Data are mean \pm SD. ¹“Outcome measures” refers to the change in values of measures of interest between baseline and week 12. β (difference in the mean outcome measures between treatment groups (probiotic group = 1 and placebo group = 0)). ²Obtained from multiple regression models. FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment insulin resistance; HDL cholesterol, high-density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; LDL cholesterol, low-density lipoprotein cholesterol; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; VLDL cholesterol, very low-density lipoprotein cholesterol; TAC, total antioxidant capacity; MDA, malondialdehyde.

and while playing games [58]. In a meta-analysis of 10 clinical trials, it was shown that probiotics had a marginal effect on mood in general [59]. In addition, no significant improvement was observed in patients with schizophrenia following treatment with *Lactobacillus rhamnosus* and *Bifidobacterium* supplements for 14 weeks [60]. These discrepancies might be due to differences in the strains used or the length of the studies. The exact mechanism of action of probiotics in the brain, and its effects on mental health disorders, has yet to be fully understood. It has been posited that the administration of probiotics may improve psychological symptoms by increasing plasma tryptophan levels, decreasing serotonin metabolite concentration in the frontal cortex, and decreasing dopamine metabolite concentrations in the amygdale [61].

4.2. The Effects of Probiotics on Lipid Profiles and Insulin Metabolism. Impaired insulin metabolism, hypertension, weight gain, and changes in serum lipids in patients receiving MMTPs may lead to long-term side effects, metabolic abnormalities, infections, chronic obstructive pulmonary disease, type 2 diabetes, and higher mortality [62–64]. Therefore, probiotics may be useful for lipid and blood glucose disorders due to their beneficial effects on lipid and glycemic parameters. Our study showed that consuming probiotics for 12 weeks in patients receiving MMTPs significantly reduced blood sugar, total cholesterol, and LDL cholesterol compared with placebo, but had no significant effect on insulin levels, HOMA-IR, QUICKI, triglycerides, and VLDL and HDL cholesterol. Although the information on the effects of probiotics on lipid and blood glucose profiles in patients receiving MMTPs is limited, several studies have evaluated these profiles in patients not receiving MMTPs. In 2019,

Kijmanawat et al. studied GDM and controlled diet in 57 pregnant women. After four weeks of probiotic use, a significant improvement in fasting blood sugar and insulin and insulin resistance levels was observed [65]. In a meta-analysis in 2020, nine studies on the effects of probiotics on neurological diseases were analyzed, showing that probiotics had a significant positive effect on insulin, insulin resistance, triglycerides, and VLDL and HDL cholesterol [21]. In another study in patients with nonalcoholic fatty liver disease, liver enzymes and clinical signs were significantly reduced after 3 months of probiotic use, although no significant changes in triglyceride levels were observed. However, contrary to the results of our study, in this study no significant change in fasting blood sugar was reported [66]. The beneficial effects of probiotics on insulin sensitivity may be due to their effects on the expression of genes associated with fat accumulation and obesity. In addition, the production of short-chain fatty acids (SCFAs) following probiotic use, especially butyrate, stimulated the release of glucose-like peptide (GLP-1) from L intestinal cells and improved glucose tolerance.

4.3. The Effects of Probiotics on Inflammatory Biomarkers and Oxidative Stress. In patients receiving MMTPs, there is a loss of antioxidant capacity and an oxidative imbalance, reflected by increased reactive oxygen species (ROS) generation, matrix metalloproteinase activity, and inflammatory cytokines [67, 68]. Therefore, probiotics may have beneficial effects on inflammatory profiles and oxidative stress. This study showed that probiotic use for 12 weeks in patients receiving MMTPs significantly decreased serum hs-CRP levels and increased plasma levels of total antioxidants and glutathione compared with the placebo group, but had no effect on plasma nitric oxide and MDA levels. Probiotics had

inhibitory effects on several oxidative stress factors in nervous system disorders in both human [17] and animal models [69–71]. Probiotics improved oxidative stress indices, such as SOD and MDA, in an animal model of Alzheimer's disease [70]. In another study in Alzheimer's disease patients, administration for 12 weeks of probiotics containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* reduced MDA, but had no effect on TAC and GSH levels [72]. In a meta-analysis of 12 studies in 2020, Amirani et al. examined the effects of probiotics on metabolic factors in patients with psychiatric disorders, showing beneficial effects on CRP, IL-10, and MDA levels [20]. In addition, 12 weeks of probiotics and vitamin D administration in patients with schizophrenia increased total antioxidants and decreased MDA and hs-CRP [73]. Probiotics decrease oxidative stress via several mechanisms. These include direct antioxidant capacity, production of antioxidant metabolites, stimulation of host antioxidant activity, increased activity of antioxidant enzyme levels by acting on NF- κ B and Nrf2 gene expression, and decreased activity of ROS-producing enzymes such as NADPH oxidase [74–76]. In addition, probiotics may affect the expression of cytokines and inflammatory chemokines by acting on TLR receptors and cascading pathways and by acting on genes essential to the inflammatory mechanisms, such as NF- κ B [77].

4.4. Study Limitations. Long-term interventions may have better effects on other metabolic profiles, anxiety, and sleep quality. Also, we did not evaluate the dietary intakes of study participants; however, we requested the participants not to change their regular dietary intakes and physical activity. In addition, we did not evaluate craving, pain, urinary profiles, and relapses in patients under MMTPs.

5. Conclusion

Overall, our novel findings suggest that patients receiving MMTPs and undergoing 12-week probiotic administration experienced improvements in symptoms of depression, but no change in anxiety and sleep quality. In addition, our study showed that probiotics had beneficial effects on blood sugar, total cholesterol, LDL cholesterol, serum hs-CRP levels, total antioxidants, and plasma glutathione, no effect on insulin, HOMA-IR, QUICKI, triglyceride, HDL and VLDL cholesterol, and plasma dialdehyde and nitric oxide levels. Further evidence is needed to demonstrate the efficacy of probiotics in patients receiving MMTPs, better characterizing their mode of action.

Abbreviations

FPG:	Fasting plasma glucose
GSH:	Total glutathione
HOMA-IR:	Homeostasis model of assessment insulin resistance
HDL cholesterol:	High-density lipoprotein cholesterol
Hs-CRP:	High-sensitivity C-reactive protein

LDL cholesterol:	Low-density lipoprotein cholesterol
NO:	Nitric oxide
QUICKI:	Quantitative insulin sensitivity check index
VLDL cholesterol:	Very low-density lipoprotein cholesterol
TAC:	Total antioxidant capacity
MDA:	Malondialdehyde
BDI:	Beck Depression Inventory
BAI:	Beck Anxiety Inventory
PSQI:	Pittsburgh Sleep Quality Index.

Data Availability

The datasets generated and/or analyzed during this study are not publicly available because the intellectual property is owned by the funding body. They may be available from the corresponding author on reasonable request containing the approval from the associated funding body.

Ethical Approval

The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences “approval no. IR.KAUMS.MEDNT.REC.1398.116.” All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Consent

At the beginning of the questionnaire distribution session, the purpose of the study was explained to the participants, and they were assured about the anonymity and confidentiality of their responses. All participants gave their signed written informed consent letters.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

NM, MRA, HM, and AGH contributed in design, conception, and statistical analysis. AHM, HRB, MV, MH, ARS, SAM, PM, and AGH contributed in data collection and manuscript drafting.

Acknowledgments

This study was the doctoral thesis on General Medicine. It has been registered with the IRCT20170420033551N9 registration code in the Iranian Center for Clinical Trials and was supported by the Clinical Research Development Unit of Kashan Shahid Beheshti Hospital, KAUMS, Iran (KAUMS/98163). The authors are thankful to all people who participated in this project. The research grant was provided by the Research Deputy of Kashan University of Medical Sciences (KAUMS/98163).

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