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Effect of a seven-strain probiotic on dietary intake, inflammatory markers, and T-cells in severe traumatic brain injury patients: A randomized, double-blind, placebo-controlled trial

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Abstract

Background Inflammatory processes are key factors in pathological events associated with severe traumatic brain injury (STBI). The aim of this trial was to determine the effect of probiotics on anthropometric measures, disease severity, inflammatory markers, and T cells in patients with STBI.

Methods Forty adult patients with STBI were enrolled in this parallel randomized, double-blind, placebo-controlled trial. Energy and protein status, Acute Physiology and Chronic Health Evaluation (APACHE II) score, Sequential Organ Failure Assessment (SOFA), interleukin 10 (IL-10), interleukin 1β (IL-1β), tumor necrosis factor-alpha (TNF-α), transforming growth factor beta (TGF-β), T-helper 17 (Th17), and T- Regulator (T-reg) cells were assessed at baseline (day 1), and week 2 (day 14) for each patient.

Results Probiotic supplementation led to a substantial reduction in the serum levels of TNF-α (from 10.15 ± 6.52 to 5.05 ± 3.27) ($P = 0.034$), IL-1β (from 11.84 ± 7.74 to 5.87 ± 3.77)

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($P < 0.001$), and Th17 cells (from 5.19 ± 1.69 to 2.67 ± 1.89) ($P < 0.001$) and a substantial increase in the serum levels of IL-10 (from 3.35 ± 1.45 to 6.17 ± 2.04) ($P = 0.038$), TGF-β (from 30.5 ± 15.27 to 46.25 ± 21.05) ($P < 0.001$), and T-reg cells (from 2.83 ± 1.43 to 4.29 ± 1.89) ($P < 0.001$) compared with the placebo group. Furthermore, no notable changes were observed in energy and protein intake and also, terms of SOFA and APACHE II scores following probiotic treatment compared with the placebo.

Conclusions Probiotics could reduce inflammation and improve cellular immunity and may be considered as an adjunctive therapy in STBI patients.

Keywords

Probiotics, cytokines, T-Lymphocytes, sever traumatic brain injury, randomized clinical trial

Introduction

A severe traumatic brain injury (STBI) is typically defined as an injury to the brain which involves one or more of the following characteristics: The Glasgow Coma Scale (GCS) score of 3 to 8, prolonged loss of consciousness, significant post-traumatic amnesia, neurological deficits, and structural damage to the brain diagnosed by imaging findings.[1] Despite advancements in medical care, the pathophysiological mechanisms underlying TBI remain complex and multifaceted, often leading to secondary injury cascades characterized by neuroinflammation, oxidative stress, and immune dysregulation.[2]

The inflammatory response following TBI is a dynamic process mediated by the release of damage-associated molecular patterns, triggering the activation of resident microglia and infiltrating peripheral immune cells, leading to the production of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α).[3] While acute inflammation serves as a protective mechanism to clear cellular debris and initiate tissue repair, prolonged and dysregulated inflammation can exacerbate neuronal damage and contribute to secondary brain injury.[4] T cells, a subset of lymphocytes crucial for adaptive immunity, play a dual role in TBI pathophysiology. While regulatory T cells (Tregs) exert immunosuppressive effects and promote tissue repair, effector T cells, such as Th1 and Th17 cells, contribute to neuroinflammation and tissue damage.[5] Imbalance in T cell subsets has been implicated in the progression of TBI pathology,[6] highlighting the potential therapeutic relevance of interventions targeting T cell responses.

Various alternative medicines have been proposed in the treatment of TBI, such as mineral supplements and various vitamins.[7] Probiotics, live microorganisms that confer health benefits when administered in adequate amounts, have garnered increasing attention for their potential therapeutic roles beyond gastrointestinal health. Probiotics exert their immunomodulatory effects through various mechanisms, including the regulation of gut microbiota composition, enhancement of intestinal barrier function, and modulation of systemic immune responses.[8] By interacting with gut-associated lymphoid tissue and the enteric nervous system, probiotics can influence immune cell trafficking, cytokine production, and T cell differentiation, thereby exerting distant effects on systemic inflammation and immune homeostasis.[9] The evidence illustrating that probiotics interact with enterocytes and dendritic cells, Th17 and T-reg, modulating adaptive immunity,[10] producing anti-inflammatory metabolites,[11,12] reducing Th17,[13] and stimulating the differentiation of anti-inflammatory T-reg cells in the gut.[14]

Various clinical trial studies with small sample sizes have investigated the effect of different strains of probiotics on STBI. Tan et al. reported that *Bifidobacterium longum*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* could improve the deviated Th1/Th2 response induced by STBI.[15] Numerous studies have investigated the anti-inflammatory effects of probiotic in TBI.[16–18] However, according to our knowledge, there is no clinical trial study investigating the effect of probiotic on Th17/T-reg balance in STBI, considering the serum level of IL-1β and TNF-α as the inflammatory cytokines and inhibitory cytokines including transforming growth factor-β (TGF-β) and interleukin-10 (IL-10). To test this hypothesis, we designed a randomized, double-blind, placebo-controlled study to provide novel insights into the immunomodulatory effects of probiotics in the context of STBI.

Methods

Study design

This parallel double-blind, placebo-controlled, randomized clinical trial with 1:1 ratio rate was performed from December 2020 to November 2021. Investigators, caregivers, and relatives of the patients were blinded to study allocation. Participants did not need to be blinded due to low consciousness. Patients with STBI admitted into two ICUs of Imam Reza Hospital and Shohada Hospital of Tabriz University of Medical Sciences (TUMS) were enrolled in this study. The study was approved by the Ethics Committee of TUMS (IR.TBZMED.REC.1399.503). This study was a part of a clinical trial registered at the Iranian Registry of Clinical Trials (Code No. IRCT20100209003320N18). This research complies with the Declaration of Helsinki standards and current ethical guidelines. After a full explanation of the study aims and protocol, written informed consent was signed by one of the first-degree relatives of the patients.

The primary outcomes of this study were changes in the Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE-II) scores, serum level of IL-1β, IL-10, TGF-β, TNF-a, and cell number of Th17 and T-reg. Secondary outcomes were changes in anthropometric indices and dietary intakes. The inclusion criteria were as follows: (1) age between 18 and 60 years; (2) suffering from STBI (defined with one or more of the following characteristics: the GCS score of 3 to 8, prolonged loss of consciousness, significant post-traumatic amnesia, neurological deficits, and structural damage to the brain diagnosed by imaging findings[1]); (3) expected ICU stay of at least 14 days; (4) GCS of < 8; (5) receiving enteral nutrition; and (6) APACHE II score of 15–30. (1) Patients with (1) pregnancy or lactating; (2) unstable hemodynamics; (3) intestinal obstruction; (4) intestinal ischemia; (5) short bowel syndrome; (6) pancreatitis and receiving total parenteral nutrition for more than 2 days; and (7) treated by immunosuppressive medications were excluded.

Sample size

Based on the mean difference of IL-10 (108.81 in the probiotic group and 76.1 in the placebo group) and standard deviation (SD) difference reported by Tan et al.,[15] power

of 80% in two-sided tests, and α=0.05, the minimum sample size to reach this significant effect size was calculated 20 patients in each group. The sample size increased to 23 patients per group by considering a 15% dropout rate.

Randomization, intervention, and allocation

Forty-six patients with STBI were randomly assigned in two groups (1:1) as "Probiotic group" and "Placebo group" using the blocking method. The four-sized blocks stratified according to gender and age groups (18–40 and 40–60 years) were created by STATA 16.0 statistical software (Stata Corp, College Station, TX) and identified by the letters A, B, C, D. The patients in the placebo group ($N=23$) received four placebo capsules (Zist Takhmir, Iran) a day (at 6-h intervals) containing maltodextrin, silicon dioxide, microcrystalline cellulose, and sodium starch glycolate, while those in the probiotic group ($N=23$) received four seven-strain probiotic capsules (10^{10} CFU) (at 6-h intervals) (Zist Takhmir, Iran) a day containing *Lactobacillus* (including *rhamnosus*, *casei, acidophilus, bulgaricus*) and *Bifidobacterium* (including *bruhe and longum*) and *Streptococcus. thermophiles* for two weeks. Because inflammatory responses are different in different phases of stress, a period of two weeks was chosen to evaluate the effect of probiotics in the initial and catabolic phases of stress.[19] Capsules were administered through enteral feeding with nasal gastric tube.

Probiotic and placebo capsules were identical in terms of smell, taste, shape, color, and size to ensure allocation concealment. At the beginning of the study, the cans containing the relevant capsules were coded by a third person other than the researchers, so that the researchers did not know the type of capsules received by each group. Investigators, caregivers, and relatives of the patients were blinded to study allocation. Participants did not need to be blinded due to low consciousness. Each patient was assigned an order code and received the capsules based on their codes. The degree of compliance was considered 90%.

Anthropometric measurements

In this study, a personal questionnaire was completed for each patient. Anthropometric measures including weight, height, body mass index (BMI), mid-upper-arm circumference (MUAC), and triceps skinfold thickness (TSF) were measured. First, height (Ht) was estimated based on ulna length and MUAC was measured on midway between the tip of the shoulder and the tip of the elbow using a non-stretchable tape measure, and then weight (Wt) was calculated using the following formula[20]:

For women: Wt (kg) = −64.6 + 2.15*MUAC (cm) + 0.54* Ht (cm)

For men: Wt (kg) = −93.2 + 3.29*MUAC (cm)+ 0.43* Ht (cm).

BMI was calculated using the following formula: BMI = Wt (kg)/[Ht (m)]^2. TSF was measured with a Harpenden skinfold caliper (UK). The calculation of mid-upper arm muscle circumference (cm) was done using the formula MUAC−(3.14 × TSF).[21]

Nutritional assessment

The nutritional status as well as the microbial quality of the enteral nutrition solutions were evaluated before the start of the intervention.[22] Interventions were designed by an expert

nutritionist based on an advanced nutritional assessment based on 25–30 kcal/kg and protein requirements as 1.2–1.5 g/kg for formula intake in patients receiving enteral tube feeding in the ICU.[23] Nutritional information of the patients was collected by a nutritionist to provide information such as nutritional status, nutritional needs, and energy intake, and setting the right time to start or continue enteral nutrition.

Assessment of disease severity

The severity of disease was assessed using the SOFA and APACHE-II scores on days 1 and 14. Calculation of the SOFA[24] and APACHE-II scores[25] was previously described.

Biochemical analysis

Fasting blood samples (10 ml) were taken on days 1 and 14. To measure cytokine levels, 5 ml of the venous blood samples were centrifuged to collect the serum samples, and then were refrigerated at −80 °C until use for ELISA. Interleukin levels were quantified using ELISA kits for IL-1β (catalog number: MBS2702034), IL-10 (catalog number: MBS825026), TGF-β (catalog number: MBS9135774), and TNF-a (catalog number: MBS824724) following the manufacturer's instructions (MyBioSource, USA).

Staining of cells for flow cytometry

Five ml of venous blood was collected at baseline and day 14 in tubes containing heparin. Th17 and T-reg cell number assessments were performed by flow cytometry. For stimulation, freshly collected PBMCs were incubated for 5 h with ionomycin (1 μg/ml) and PMA (25 ng/ml) in the presence of monensin, with the aim of enhancing the intracellular IL-17A stain (1.7 μg/ml). For cell surface markers staining, an antibody (anti- Human CD4) was added to a 100 μL aliquot of whole blood. The cells were incubated for 30 min at 2–8 °C, in the dark. Then, cells were washed with 2 mL Flow Cytometry Staining Buffer twice and were centrifuged at 300 g for 10 min at 4 °C. Ultimately, the supernatant was discarded and re-suspended in 500 μL Flow Cytometry Staining Buffer, incubated for 30 min at 2–8 °C temperature, protected from light. After staining, the cells were washed and re-suspended in a cell staining buffer before to being read by a flow cytometry instrument. At least a concentration of 1×10^6 cells was used for the flow cytometry procedure.

Statistical analysis

All statistical analyses were performed using SPSS software (IBM SPSS Statistics, Armonk, USA, ver. 25). The intention-to-treat (ITT) method was applied on all data analyses performing multiple imputation methods. Kolmogorov–Smirnov test was used to check the distribution of continuous variables. Symmetric continuous data were presented as mean ± SD. Qualitative data were presented as frequency (%). Between-group comparison was performed using an independent samples t-test. Moreover, paired samples t-test was used for intra-group comparisons. The between-group differences in qualitative data were assessed using the

Chi-square test. Analysis of covariance (ANCOVA) was performed to adjust the effects of confounders on the final effect size. The statistically significant level was considered P-value less than 0.05.

Results

Baseline characteristics

Forty out of forty-six subjects completed the study (CONSORT flowchart is presented in **Figure 1**). However, due to ITT method, all 46 data were analyzed. Three patients from each placebo and the probiotic group were excluded due to food intolerance, gastrointestinal bleeding, death, and discharge from the ICU before day 14, during the study (unrelated to the intervention). The baseline characteristics of the patients are presented in **Table 1**. There were no substantial differences within- and between-groups in terms of gender, age, weight, BMI, MUAC, TSF, and GCS scores ($P > 0.05$). However, probiotic supplementation led to a significant improvement in GCS score compared to the placebo group ($P < 0.001$).

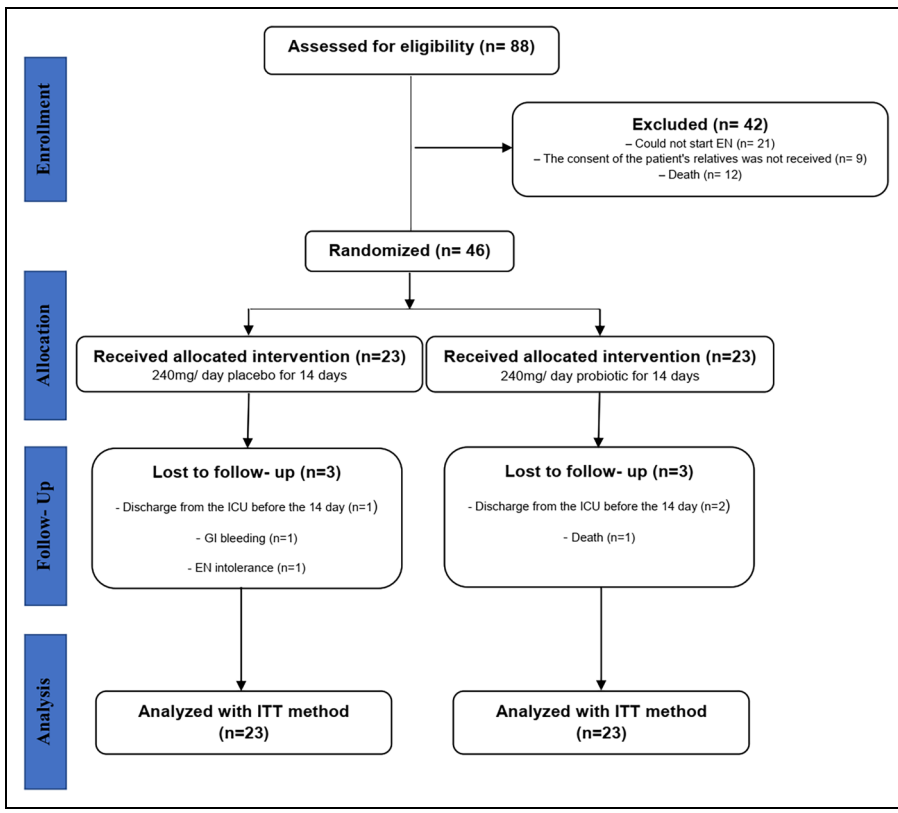

Figure 1. Study flow chart (EN: enteral nutrition).

Table 1. Changes in demographic, anthropometric measures, and GCS in both groups.

Variable	Probiotic ($n = 23$)	Placebo ($n = 23$)	*P*
Age (year)	33.50 ± 13.20	31.3 ± 15.96	0.613††
Gender (%)			
Male	16 (70)	14 (61)	0.536†††
Female	7 (30)	9 (39)	
Weight (kg)			
Baseline	72.80 ± 15.63	66.85 ± 11.62	0.18††
Week 2	72.95 ± 15.41	66.65 ± 11.66	0.892††††
$P^{\dagger}$	0.379	0.104	
BMI (kg/m^2)			
Baseline	23.58 ± 4.35	23.60 ± 4.04	0.986††
Week 2	23.79 ± 4.24	23.53 ± 4.00	0.72††††
$P^{\dagger}$	0.669	0.757	
MUAC (cm)			
Baseline	27.85 ± 3.39	27.30 ± 3.31	0.607††
Week 2	27.90 ± 3.38	27.25 ± 3.34	0.873††††
$P^{\dagger}$	0.33	0.33	
TSF (cm)			
Baseline	17.95 ± 4.94	16.10 ± 5.41	0.267††
Week 2	17.96 ± 4.95	16.11 ± 5.42	0.952††††
$P^{\dagger}$	0.33	0.33	
GCS (score)			
Baseline	5.15 ± 1.22	5.90 ± 1.55	0.098††
Week 2	6.65 ± 1.70	5.85 ± 1.39	**<0.001**††††
$P^{\dagger}$	0.071	0.092	

For the values that have a substantial difference, we have bolded the *p* values to make the significance obvious.
All continuous variables are reported as mean ± SD.
†: Paired t-test.
††: Independent t-test.
†††: Chi-squared test.
††††: ANCOVA test adjusted by baseline values.

Nutritional assessments

Nutritional assessment of patients in each group were assessed and shown in **Table 2**. Mean protein intakes were compared with protein requirements calculated by formulaic methods. The most common reasons for interrupting enteral nutrition included temporary cessation for medical procedures or increased gastric residuals to more than 200 ml. No substantial differences were reported between two groups for mean energy and protein intake ($P > 0.05$).

Cytokines and T-cells

Table 3 shows that probiotic led to a significant decrease in the serum levels of pro-inflammatory cytokine ($P = 0.034$ for TNF-α and $P < 0.001$ for IL-1β) and increase in the serum levels of anti- inflammatory cytokine ($P = 0.038$ for IL-10 and $P < 0.001$

Table 2. Nutritional variables of patients by treatment group at the baseline.

Variable	Probiotic ($n = 23$)	Placebo ($n = 23$)	$P^{\dagger}$
Energy intake (kcal/d)	$1795.45 \pm 262.30^{\dagger}$	1665 ± 225.45	0.088
Energy requirements met (%)††	95.12 ± 4.22	98.37 ± 4.60	0.074
Protein intake (g/d)	75.47 ± 3.69	72.20 ± 4.23	0.062
Protein requirements met (%)†††	86.45 ± 2.42	79.29 ± 1.93	0.054

All continuous variables are reported as mean ± SD.
††: Independent t-test.
††: Determined by energy intake from enteral nutrition/energy requirements from formulaic assessment of 25 kcal/kg/d.
†††: Determined by g protein consumed via enteral nutrition/grams protein required from formulaic assessment of 1.2–1.5 g/kg/d.

for TGF-β) compared to the placebo group. Regarding T-cell numbers, probiotic significantly elevated T-reg cells and decreased Th17 cells ($P < 0.001$ for both) compared to the placebo (**Table 3**).

SOFA and APACHE II

Figure 2 reveals changes in clinical status based on SOFA and APACHE II scores. There were no significant differences at the baseline and endpoint ($P > 0.05$).

Adverse effect

There were no reported serious adverse events leading to trial discontinuation following probiotic supplementation.

Discussion

The present randomized, double-blind, placebo-controlled trial was designed for the first time to determine the effects of seven-strain probiotic supplementation on the immune response and clinical outcomes of patients with STBI. We conducted our study in a trauma ICU on patients who had sustained only one STBI, thus reducing heterogeneity, which has been an important issue in ICU studies. Furthermore, since low-grade chronic inflammation in elderly subjects may impair the therapeutic effects of probiotic consumption, we did not include subjects over 60 years of age.[26]

Our results showed a decrease in IL-1β, TNF-α, and Th17 cells, as well as an increase in IL-10, TGF-β, T-reg cells, and GCS, but no significant difference was observed in SOFA and APACHE II scores following probiotic supplementation compared to the placebo group. Noshadi et al. in a meta-analysis of seven studies showed that probiotic/synbiotic supplementation had no significant effect on C-reactive protein, IL-6 and ICU length in TBI and multiple trauma patients.[27] Only three of seven studies prescribed probiotic[15,17,18] on TBI patients. However, their findings had low-to-moderate certainly of evidence. Moreover, unlike our study, which used seven probiotic strains,

Table 3. Changes in T-helper cells and cytokines in the probiotic and placebo groups.

Variable	Probiotic group ($n = 23$)	Placebo group ($n = 23$)	P ††	P †††
TNF-α (pg/ml)				
Baseline	10.15 ± 6.52	9.71 ± 6.11	0.441	**0.034**
Week 2	5.05 ± 3.27	7.96 ± 4.16	**0.027**	
Mean change (95% CI) †	−5.1 (−6.8, −3.39)	−2.25 (−3.05, 1.45)		
P ††††	**<0.001**	0.069		
IL-1β (pg/ml)				
Baseline	11.84 ± 7.74	11.12 ± 7.12	0.759	**<0.001**
Week 2	5.87 ± 3.77	8.64 ± 4.58	**<0.001**	
Mean change (95% CI)	−5.97 (−8.29, −3.66)	−2.48 (−4.30, 0.65)		
P	**<0.001**	0.051		
IL-10 (pg/ml)				
Baseline	3.35 ± 1.45	3.64 ± 1.52	0.541	**0.038**
Week 2	5.17 ± 2.04	4.51 ± 1.70	**0.049**	
Mean change (95% CI),	1.82 (1.42, 2.23)	0.87 (0.42, −1.33)		
P	**<0.001**	0.061		
TGF-β (pg/ml)				
Baseline	30.5 ± 15.27	30.15 ± 17.06	0.946	**<0.001**
Week 2	46.25 ± 21.05	36.95 ± 19.11	**0.004**	
Mean change (95% CI)	15.75 (12.38, 19.12)	6.8 (1.65, −.95)		
P	**<0.001**	0.052		
Th17 cells (%)				
Baseline	5.19 ± 1.69	5.05 ± 1.46	0.781	**<0.001**
Week 2	2.67 ± 1.89	4.45 ± 1.49	**0.003**	
Mean change (95% CI)	−2.53 (−1.90, −1.14)	−0.61 (−1.06, 0.16)		
P	**<0.001**	0.051		
T-reg cells (%)				
Baseline	2.83 ± 1.43	2.95 ± 1.55	0.801	**<0.001**
Week 2	4.29 ± 1.89	3.40 ± 1.48	**0.007**	
Mean change (95% CI)	1.46 (0.97, 1.91)	0.45(−0.08, 0.99)		
P	**<0.001**	0.095		

For the values that have a substantial difference, we have bolded the p values to make the significance obvious. All continuous variables are reported as mean ± SD.

†: Mean change (95% CI) of within group.

††: Independent t-test shows the difference of the variables between two groups at the baseline and endpoint.

†††: ANCOVA test adjusted by baseline values.

††††: Paired t-test.

other studies prescribed limited strains of probiotics. Therefore, it seems that more studies on various inflammatory and immune system biomarkers with appropriate design are necessary to clarify the anti-inflammatory and immunomodulatory effects of probiotics in TBI patients.

Regarding cytokines, previous studies reported different results, some of which were consistent and in some cases inconsistent with our results. So far, no clinical study has investigated the effect of probiotics on TGF-β in TBI. However, beneficial effect of probiotic on TGF-β has studied in other health conditions.[28,29] Anti-inflammatory effects of TGF-β in the late phase of stress and its role in tissue remodeling can accelerate the

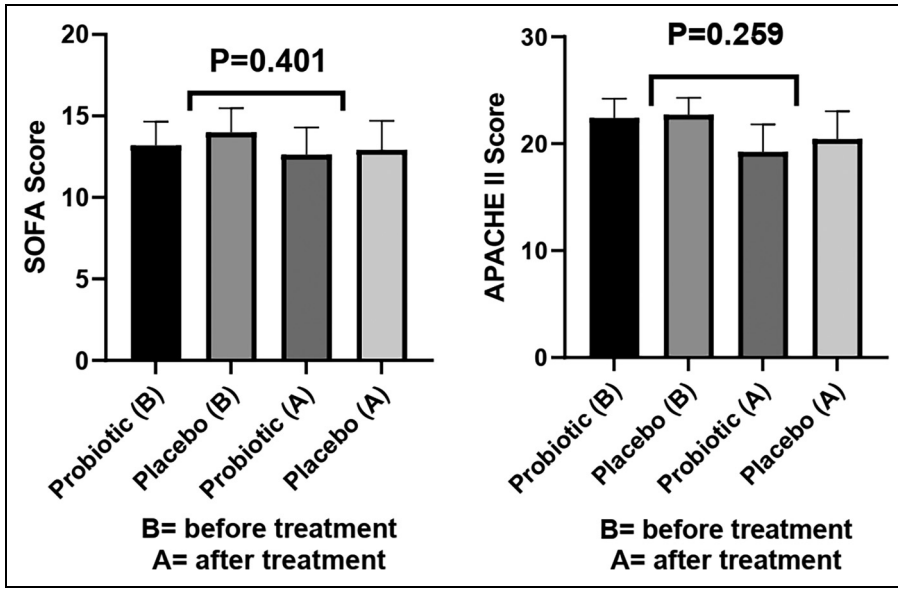

Figure 2. APACHE II and SOFA scores over the treatment period in the probiotic and placebo groups.

recovery of damaged tissue. However, TGF-β can exert inflammatory responses in the early phase.[30] Tan et al.[15] and Wan et al.[18] reported that probiotic significantly decreased IL-10 level in TBI patients following 21 and 15 days of supplementation. However, Tan et al. showed that probiotic in the first week and partly in the second week had increasing effect on IL-10 level.[15] It has been shown that IL-10 up-regulation and down-regulation has beneficial effects in the early and middle phase of stress, respectively.[31] Increasing effect of probiotic on IL-10 level in our study in the catabolic phase can limit the extent of immune responses and prevent excessive inflammation and immune-mediated damage. Regarding TNF-α, our result was consistent with results reported by Wan et al.[18] Previous studies showed that probiotics administration decreased not only the gene expression of TNF-α in peripheral blood mononuclear cells[32] but also serum levels of pro-inflammatory cytokines such as TNF-α and IL-1β.[33] However, Brenner et al. reported that probiotic had no significant effect on IL-1β.[17] This study prescribed only *Lactobacillus reuteri*, which led to different results compared to studies prescribing multi-strain probiotics. Cytokines like TNF-α, IL-1β, and IL-17A are crucial cytokines in TBI that stimulate the inflammatory cascade with worse outcomes and focus on more morbidity and mortality.[34] Previous studies have shown that increased inflammatory cytokine production is important prognostic factors of poor clinical outcomes and recovery in TBI patients.[35] Improving effect of probiotic on GCS in our study can indicate the positive effect of probiotics on recovery in STBI patients. Similarly, Wan et al. showed that probiotic improved GCS in patients with STBI.[18] In a meta-analysis study, Yi et al. reported that probiotic led to a significant improvement in risk of mortality and decrease in ICU stay in STBI.[36]

Nutritional support and improvement of epithelial barriers are critical concerns in intensive care for patients with TBI. In 2007, early feeding guidelines, especially within 48-h post-injury for patients with severe TBI to reduce clinical malnutrition, prevent bacterial translocation from the gastrointestinal tract, improve GCS (especially those with a GCS score of 6–8), enhance the immune status and feeding tolerance, and improve overall outcome in STBI patients were released. Although no changes in energy and protein intake were observed between the two groups in the present study, it is possible that increasing the sample size and increasing the duration of the study can have beneficial effects on the outcome of TBI patients.[37]

Th17 cells and T-reg are a subset of CD4+ T cells that play an important role in promoting (Th17) or suppressing (T-reg) inflammation and are crucial in the regulation of immunity and inflammation.[38] T-reg cells were inversely correlated with the rate of inflammation progression in TBI patients, and it has been recently suggested that T-reg cell therapy can be considered as a potential immunotherapy approach in TBI.[39] Th17 cell activation has recently been reported in a mouse model of TBI, and this is increased after motor nerve injury. Therefore, one hypothesis is that due to brain blood barrier damage, the priming of specific Th17 cells and their recruitment into the CNS promotes specific inflammation and leads to cell death.[40] The effect of probiotics on Th17 and T-reg cells has not yet been extensively studied, however, the results of previous studies have shown that the precise effects of probiotics on T cells are still unclear.[41] A number of studies have shown that probiotic supplementation suppresses Th17 response,[42,43] while in other studies there was no significant difference in the mean number of T-reg cells.[44] Furthermore, prolonged T-reg cell suppression is associated with an increased risk of infectious complications and reduced immune function in patients with brain injury.[45] Despite the heterogeneous results, it cannot be concluded that probiotics are not useful as adjunctive immunomodulatory agents. Therefore, further studies in human models with different inflammatory conditions and different probiotic strains are needed.

Elevated APACHE II score—as a measure of disease severity—has been reported to be closely associated with an increased risk of many common illnesses and in-hospital death.[46] In addition, the SOFA score is a simple and objective score that allows estimation of the number and severity of organ dysfunction and is also a good indicator of prognosis in the first few days of ICU admission.[47] Independent of the initial score, an increase in SOFA score during the first 48 h in the ICU predicts a mortality rate of at least 50%.[48] In a study among patients with STBI, APACHE II and SOFA scores were not significantly affected by probiotic treatment.[15] It seems that long-term treatment may be the way to evaluate the clinical effects of probiotic administration in the ICU. It may also be affected by the probiotic strains and dosage as well as the condition of the patients.

Limitations and strengths

Although this study has limitations such as not considering the effect of commonly used drugs, the relatively short duration of the study, and the synergistic effect of the strains used, the strengths of our study include the high dose and administration of multi-strain probiotics, as well as the survey of patients from two ICU centers. Moreover, this study was

registered at the Iranian Registry of Clinical Trials (Code No. IRCT20100209003320N18). Our results revealed that daily administration of seven-strain probiotics as adjunctive therapy in patients with STBI is associated with improvements in inflammation. As there is not enough evidence supporting the routine use of probiotics in STBI patients, more clinical trials with long-duration therapy, various types of probiotics, and a larger sample size are required.

Conclusion

Probiotics could reduce inflammation by decreasing IL-1β, TNF-α, and Th17 cells and increasing IL-10, TGF-β, T-reg cells may be considered as an adjunctive therapy in STBI patients. Seven-strain probiotic can improve GCS in STBI patients.

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Author contributions

S. H. A. and M. E. M. designed the study, collected the data, analyzed the data, drafted, and prepared the manuscript. M. Y. designed the study, collected the data, and critically revised the manuscript. S. R. A. collected the data and critically revised the manuscript. A. M. designed the study and edited the manuscript.

Declaration of conflicting interests

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