ORIGINAL ARTICLE

Effect of a multispecies probiotic on inflammatory markers in critically ill patients: A randomized, double-blind, placebo-controlled trial

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Background: Impairment of intestinal barrier function and increased translocation of bacteria to the systemic blood flow contribute to the emergence of sepsis. Probiotics might be of beneficial effects on critically ill-patients, modulating intestinal barrier function and reducing inflammation. The aim of this trial was to determine the effect of probiotics on inflammatory markers in critically ill-patients in Intensive Care Unit (ICU). **Materials and Methods:** This trial was conducted on 40 critically ill-patients admitted to the ICU. Patients were randomly assigned to receive placebo or probiotic containing *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophilus* (VSL#3) for 7 days. Acute Physiology and Chronic Health Evaluation (APACHE II) score Sequential Organ Failure Assessment (SOFA) and systemic concentrations of interleukin-6 (IL-6), procalcitonin (PCT) and protein C were measured before initiation of the study and on days 4 and 7. **Results:** A significant difference in IL-6 ($P = 0.003$), PCT ($P = 0.014$) and protein C ($P < 0.001$) levels, and also APACHE II and SOFA scores ($P < 0.001$) was seen over the treatment period between two groups. Moreover, there was a significant decrease in serum IL-6 levels (from 211.85 ± 112.76 to 71.80 ± 28.41) ($P < 0.001$) and PCT levels (from 1.67 ± 1.27 to 0.47 ± 0.41) ($P < 0.001$) and a significant increase in serum protein C levels (from 7.47 ± 3.61 to 12.87 ± 3.63) ($P < 0.001$) in probiotic group during the study. **Conclusion:** Probiotics could reduce inflammation in critically ill-patients and might be considered as an adjunctive therapy in the treatment of critically ill-patients.

Key words: Inflammation, probiotics, sepsis

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INTRODUCTION

Sepsis which is characterized by a systemic inflammatory response syndrome (SIRS) and the presence of an infection is one of the most serious complications in critically ill patients and it may finally lead to cell death and multi-organ dysfunction syndrome (MODS). Sepsis and its subsequent complications are the most common cause of death in Intensive Care Units (ICUs).[1-3] It is estimated to affect 18 million people worldwide annually and kill 1,400 people daily. In the United States alone, 750,000 people develop sepsis each year about 30% of which die.[4] Inflammation plays a key role in the development of sepsis; elevated levels of various inflammatory cytokines could be detected in patients with sepsis.[5] Interleukin 6 (IL-6) is an inflammatory biomarker with a diagnostic and prognostic value in patients with sepsis which is used in the prediction of mortality in patients with severe sepsis.[6-8] Procalcitonin (PCT) has been proposed as a specific biomarker for early diagnosis of sepsis in recent years that can be helpful in determining the prognosis of sepsis.[6,9] Protein C, an important anticoagulant and anti-inflammatory factor, is a prognostic indicator in sepsis, and its deficiency is associated with poor outcome.[10,11] Gastrointestinal (GI) tract has been blamed for the pathogenesis of sepsis and MODS due to the impairment of intestinal barrier function and increased translocation of bacteria to systemic blood flow.[12-15] Intestinal microbes can be a major source of systemic infection in patients of ICU.[13] In contrast, *Bifidobacterium* and *Lactobacillus*, *Endogenous* probiotic bacteria of the gut, play a critical role in maintaining the intestinal mucosal barrier and enhancing immune responses.[16] Despite the introduction of various antibacterial and antifungal drugs, pharmacological interventions have not been so successful in reducing sepsis mortality and have failed to increase the recovery rate.[17]

Probiotics are of beneficial effects in the treatment of a wide range of GI disease such as different types of diarrhea,[18,19] inflammatory bowel diseases,[20] irritable

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bowel syndrome[21] and pouchitis.[22,23] Since they play a role in reduction or elimination of pathogens and toxins, releasing of nutrients, antioxidants and growth factors to stimulate intestinal motility, and regulation of the immune defence mechanisms by changing the intestinal flora, probiotics seem to have beneficial effects in the improvement of critically ill-patients.[24,25] We have previously studied the effect of probiotics on CRP and oxidative stress factors and shown that they can have an effect on inflammation.[25] Thus, the purpose of this double-blind, placebo-controlled, and randomized clinical trial was to determine the effect of probiotic containing *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophilus* on inflammatory biomarkers in critically ill-patients in the ICU.

MATERIALS AND METHODS

Study participants

Patients admitted to the ICU of the Shohada Hospital (Tabriz University of Medical Sciences, Tabriz, Iran) between December 2011 and October 2012 was eligible for the study. Inclusion criteria were as the followings: (1) Critically ill-patients with positive SIRS and Acute Physiology and Chronic Health Evaluation (APACHE II) score of 15-30; (2) receiving enteral nutrition; (3) expected ICU stay of at least 7 days. Exclusion criteria were as the followings: (1) pregnant and lactating women; (2) patients who could not tolerate enteral nutrition; (3) unstable hemodynamics; (4) intestinal obstruction; (5) intestinal ischemia; (6) short bowel syndrome; (7) pancreatitis.

After approval of ethics committee of the Tabriz University of Medical Sciences and obtaining written informed consent from the patients or their legal guardians, 40 patients (20 in probiotic and 20 in the placebo group) were enrolled in this trial. A computer-generated random sequence was kept in a remote secure location and administered by an independent third party who was not involved with the clinical conduct of the study until all study data were collected and verified. Patients and those involved in enrolling participants, administering interventions and assessing outcomes were blind to group assignments. Our clinical trial was registered in Iranian Registry of Clinical Trials with code number of (IRCT201112143320N6).

Treatment

All patients received enteral nutrition with Fresubin original fibre (Fresenius Kabi, Homburg, Germany) throughout the first 24-h of admission via nasogastric tube, which provided 1kcal/ml. They were randomly assigned to two 20-person groups; the first group received standard treatment plus placebo, and the second group received standard treatment plus VSL#3, 2 sachets daily for 7 days. Each sachet of probiotics (VSL#3; VSL Pharmaceuticals, Sigma-Tau Pharmaceuticals Inc. Ft Lauderdale, FL) contained 450 billion viable lyophilized bacteria consisting of 4 strains of *Lactobacillus* (*Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus,* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*), 3 strains of *Bifidobacterium* (*Bifidobacterium longum, Bifidobacterium breve,* and *Bifidobacterium infantis*) and *Streptococcus salivarius* subsp. *Thermophilus*.

Study design

This was a single-center double-blind (researcher and patient) and placebo-controlled trial. Enteral feeding was started at 25 ml/h and increased by 25 ml/h every 4 h until the target rate was achieved. When gastric residual volumes exceeded 150 ml, prokinetic agents were initiated, and feeding was advanced until the target rate was achieved. The probiotic group received 2 sachets of probiotics, while the placebo group received 2 sachets of placebo twice daily at 9 a.m. and p.m. The placebo preparation had identical packing and was manufactured by the same company. All patients in the study received concomitant therapy, including antibiotics, as considered appropriate by the attending physician.

Nutritional assessment

Weight and height of the patients were recorded, and body mass index was calculated using the formula weight (kg)/height^2 (m). Energy requirements were calculated as 25-30 kcal/kg and protein requirements as 1.2-1.5 g/kg. Daily energy and protein intake were recorded.

Outcome measures

Acute physiology and chronic health evaluation II and sequential organ failure assessment scores

Acute Physiology and Chronic Health Evaluation II[26] and Sequential Organ Failure Assessment (SOFA)[27] scores were calculated for all patients prior to the study and on days 4 and 7.

Biochemical analysis

Five ml of blood was obtained from each patient to evaluate IL-6, PCT and Protein C before initiation of the study and on days 4 and 7.

Statistical analysis

The total sample size of 40 subjects (20 in each group) was calculated based on published levels of IL-6 differences in critically ill-patients[28] at the 5% significance level with the power of 80%. Samples were selected from general ICU of Shohada hospital in Tabriz using convenience sampling method.

The data were analyzed using the statistical software program Statistical Package for the Social Sciences (SPSS Inc. 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412) Version 16.0, SPSS. Kolmogorov–Smirnov test was used to assess the normality of the variables distribution.

Independent *t*-tests were performed on all baseline data between groups. Differences in variables at baseline and after treatment were assessed with a repeated measures analysis of variance that included a time × treatment interaction. Data were further analyzed with a Sidak *post-hoc* test for multiple comparisons. Independent *t*-tests were used to assess differences between the treatment groups. $P < 0.05$ were considered as significant for all statistical tests.

RESULTS

Eligibility was determined for a total of 96 ICU admissions between December 2011 and October 2012. Among eligible patients, 40 were enrolled [Figure 1]. Demographic characteristics of patients are shown in Table 1. No significant differences in age, sex, body mass index and use of antibiotics were observed between two groups.

Nutritional variables of patients in each group were assessed and shown in Table 2. Mean daily energy intake was compared with energy requirements derived from formulaic assessment of 25kcal/kg/d. Mean protein intakes were compared with protein requirements calculated by formulaic methods. No significant differences existed between two groups for mean energy and protein intake. The two most common reasons for interrupting enteral nutrition included temporary cessation for medical procedures or increased gastric residuals to more than 200 ml.

There was a significant difference in serum IL-6, PCT and protein C levels at the end of the study between two groups ($P = 0.001$, $P = 0.005$ and $P < 0.001$, respectively) [Figure 2]. Table 3 shows the levels of IL-6, PCT and protein C and also APACHE II and SOFA scores in various days of the study. There was a significant difference in IL-6 ($P = 0.003$), PCT ($P = 0.014$) and protein C ($P < 0.001$) levels, and also APACHE and SOFA scores ($P < 0.001$) over the treatment period between two groups. A significant decrease in serum IL-6 and PCT levels ($P < 0.001$) and a significant increase in serum protein C levels ($P < 0.001$) in the probiotic group were seen during the study. There was a significant decrease in APACHE II and SOFA scores in both probiotic ($P < 0.001$) and placebo ($P = 0.034$ and $P = 0.029$, respectively) groups during the study; however, the decreases were more in the probiotic group compared with placebo group. *Post-hoc* Sidak test showed a significant difference for all variables among the different days of the study in the probiotic group [Table 4].

Table 1: Demographic and baseline characteristics of patients

	Probiotic group (*n*=20)	Placebo group (*n*=20)	*P*
Age (year)	33.60±5.50*	35.60±5.03	0.238†
Male/female	13/7	14/6	0.500††
BMI (kg/m^2)	24.30±2.92	24.70±3.00	0.677†
Reason for ICU admission (*n* (%))			
Trauma	11 (55)	14 (70)	0.514††
Postoperation	9 (45)	6 (30)	
Types of antibiotics (*n*/d)	2.40±0.75	2.60±0.82	0.427†
Mechanical ventilation (*n* (%))	16 (80)	14 (70)	0.716††

†Independent *t*-test; ††Chi-square; *Mean±SD. BMI=Body mass index; ICU=Intensive care unit; SD=Standard devition

Table 2: Nutritional variables of patients by treatment group

	Probiotic group (*n*=20)	Placebo group (*n*=20)	*P*†
Energy intake (kcal/d)	1503.75±231.60*	1617.50±185.51	0.095
Energy requirements met (%)¥	84.98±3.60	87.24±3.92	0.065
Protein intake (g/d)	56.39±8.68	60.65±6.95	0.095
Protein requirements met (%)¥¥	66.38±2.82	68.15±3.06	0.065

†Independent *t*-test; *Mean±SD; ¥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 g/kg/d. SD=Standard deviation

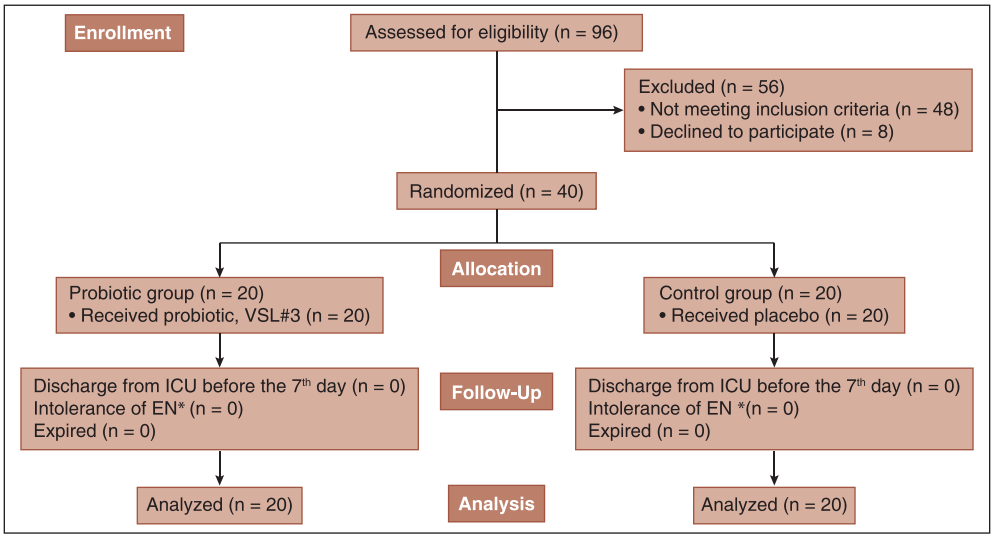

Figure 1: Study overview (*Enteral nutrition)

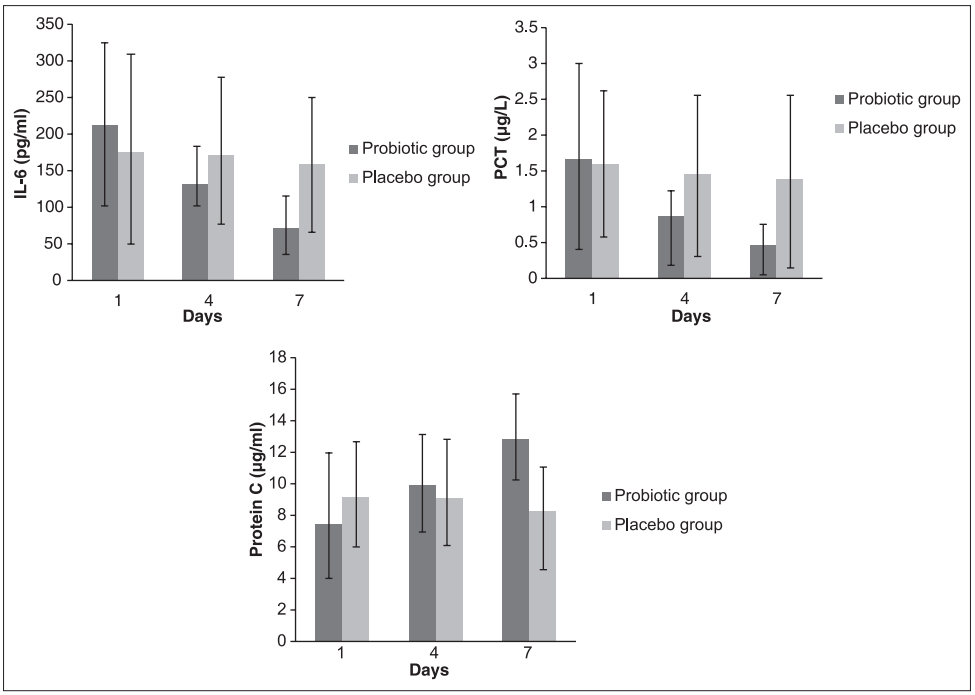

Figure 2: Serum interleukin-6, procalcitonin and protein C levels during the different days of the study †Independent *t*-test

Table 3: Serum IL-6, PCT and protein C levels and APACHE II and SOFA scores during the intervention period

Variable	Day	Probiotic group (*n*=20)	Placebo group (*n*=20)	*P*†	*P*ϕ
IL-6 (pg/ml)	1st day	211.85±112.76*	175.50±130.11	0.351	0.003
	4th day	131.70±28.42	171.20±99.45	0.150	
	7th day	71.80±28.41	159.75±94.58	0.001	
	*P*ϕϕ	<0.001	0.711		
PCT (µg/L)	1st day	1.67±1.27	1.59±1.03	0.823	0.014
	4th day	0.87±0.67	1.45±1.11	0.055	
	7th day	0.47±0.41	1.38±1.26	0.005	
	*P*ϕϕ	<0.001	0.603		
Protein C (µg/ml)	1st day	7.47±3.61	9.16±2.82	0.107	<0.001
	4th day	9.94±3.01	9.11±3.42	0.421	
	7th day	12.87±3.63	8.28±2.81	<0.001	
	*P*ϕϕ	<0.001	0.502		
APACHE II	1st day	22.80±4.73	22.45±4.57	0.813	<0.001
	4th day	17.90±5.05	19.95±4.13	0.168	
	7th day	13.85±4.82	20.85±7.55	0.001	
	*P*ϕϕ	<0.001	0.034		
SOFA score	1st day	12.25±2.57	12.55±2.60	0.716	<0.001
	4th day	9.75±2.42	11.10±2.29	0.078	
	7th day	7.50±2.01	11.30±3.78	<0.001	
	*P*ϕϕ	<0.001	0.029		

*Mean±SD; †Independent *t*-test shows the difference of the variables between two groups; ϕRepeated-measures ANOVA shows the trend of changes of the variables during the study between two groups; ϕϕRepeated-measures ANOVA shows the trend of changes of the variables during the study in each group. IL-6=Interleukin 6; PCT=Procalcitonin; APACHE=Acute physiologic and chronic health evaluation; SOFA=Sequential organ failure assessment; SD=Standard deviation

Two patients in the probiotic group and five patients in the placebo group developed sepsis during their ICU stay. Although administration of probiotics decreased the incidence of sepsis, there was not a significant difference between two groups (P = 0.407). The organisms isolated from the blood culture of the patients with sepsis were as followings:

- Pseudomonas aeruginosa: One in probiotic and two in the placebo group
- *Staphylococcus aureus*: One in probiotic and two in the placebo group
- Acinetobacter: One in the placebo group.

No patients in the probiotic group developed *Lactobacillus*-induced sepsis.

DISCUSSION

The present study used a double-blind, placebo-controlled and randomized design to determine the effects of probiotics on critically ill, eternally fed patients. Overall, the patients who received probiotic showed a greater reduction in inflammation than did the patients who received placebo. Serum levels of IL-6 and PCT have been recommended for early identification of inflammation and sepsis.[6,29] IL-6 is a 21 kDa glycoprotein produced by many cell types including lymphocytes, fibroblasts and monocytes. It has many systemic effects including induction of acute phase protein production in the liver. In clinical studies, IL-6 appears to be a good indicator of activation of the cytokine cascade and predicts subsequent organ dysfunction and mortality.[30] PCT, the precursor to calcitonin, is an 116-amino-acid protein and has been shown to be associated with inflammation and sepsis.[9] In our study, administration of probiotic significantly decreased IL-6 and PCT levels. A similar

Table 4: Mean difference in serum IL-6, PCT and protein C levels and APACHE II and SOFA scores over the treatment period in each group

	Days		Probiotic group (*n*=20) mean difference (CI: 95%)	*P*§	Placebo group (*n*=20) mean difference (CI: 95%)	*P*§
IL-6 (pg/ml)	1	4	−80.15 (−121.57,−38.72)	<0.001	−4.30 (−49.62, 41.02)	0.993
	1	7	−140.05 (−202.52,−77.57)	<0.001	−15.75 (−102.88, 71.38)	0.954
	4	7	−59.90 (−96.45,−23.34)	0.001	−11.45 (−70.40, 47.50)	0.944
PCT (μg/L)	1	4	−0.80 (−1.18,−0.41)	<0.001	−0.14 (−0.49, 0.21)	0.670
	1	7	−1.20 (−1.81,−0.59)	<0.001	−0.20 (−1.02, 0.61)	0.889
	4	7	−0.40 (−0.67,−0.14)	0.002	−0.06 (−0.74, 0.61)	0.993
Protein C (μg/ml)	1	4	2.47 (1.04, 3.89)	0.001	−0.05 (−1.88, 1.78)	1.000
	1	7	5.40 (3.76, 7.03)	<0.001	−0.88 (−3.20, 1.44)	0.706
	4	7	2.92 (1.68, 4.16)	<0.001	−0.82 (−3.17, 1.51)	0.745
APACHE II score	1	4	−4.90 (−5.86,−3.93)	<0.001	−2.50 (−3.76,−1.23)	<0.001
	1	7	−8.95 (−10.99,−6.91)	<0.001	−1.60 (−4.31, 1.11)	0.363
	4	7	−4.05 (−5.70,−2.40)	<0.001	−0.90 (−1.50, 3.30)	0.713
SOFA score	1	4	−2.50 (−3.27,−1.72)	<0.001	−1.45 (−2.09,−0.80)	<0.001
	1	7	−4.75 (−5.99,−3.50)	<0.001	−1.25 (−2.88, 0.38)	0.168
	4	7	−2.25 (−3.21,−1.28)	<0.001	0.20 (−1.22, 1.62)	0.978

§*Post-hoc* Sidak test. CI=Confidence interval; IL-6=Interleukin 6; PCT=Procalcitonin; APACHE=Acute physiologic and chronic health evaluation; SOFA=Sequential organ failure assessment

finding was reported by McNaught *et al*. who showed that enteral administration of ProViva, an oatmeal-based drink containing *L. plantarum* 299 v to critically ill patients resulted in significantly lower levels of IL-6 in the probiotic group compared with the controls.[28] In another study performed on critically ill trauma patients receiving a synbiotic formula (Synbiotic 2000Forte) or placebo, there was a statistically significant difference between groups regarding PCT levels with the synbiotic group having the lowest.[31] In a study conducted on pigs, probiotic supplementation including 30% *L. acidophilus*, 30% *Bifidobacterium lactics*, 20% *Bacillus subtilis* and 20% *Bacillus natto* did not have a significant effect on PCT concentrations of pigs compared with the control group.[32] The positive results of our study may be due to the patient population and the type of the used probiotic. Our trial was performed in a surgical ICU on patients most of whom were traumatic patients; therefore, heterogeneity which is one the most important problems in ICU population was decreased. VSL#3, contains 8 different strains of live lactic acid bacteria specially selected to produce an optimal synergistic composition of bacteria, is a potent probiotic medical food that delivers the highest available concentration of beneficial live bacteria than any other probiotic.[33,34] At the cellular level, VSL#3 positively affects a variety of substances that are involved in gut function. This is important to ensure the correct absorption of nutrients and to maintain barrier functionality.[35] Finally, evidence suggests that VSL#3 can reduce intestinal permeability by tightening the junctions between the cells in the outer layer of the intestine that in turns reduces the likelihood of translocation of pathogens from the intestine into the blood.[36]

Plasma levels of acute phase proteins involved in coagulation and fibrinolysis are elevated during inflammation while natural anticoagulant mechanisms are depressed. Protein C is a natural component of the anticoagulant system. In sepsis, activated protein C attempts to achieve homeostasis by decreasing inflammation and coagulation.[10,37] In our study, administration of probiotic significantly increased protein C levels. To the best of our knowledge, no study has been performed regarding the effect of probiotic administration on protein C.

Acute Physiology and Chronic Health Evaluation II score is a tool to measure the disease severity. An increasing score is closely correlated with the subsequent risk of many common diseases and hospital death.[25,38] SOFA score is a simple and objective score that allows calculation of both the number and the severity of organs' dysfunctions and during the first few days of ICU admission is a proper indicator of prognosis.[27] 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%.[39] In our study, both APACHE and SOFA scores improved significantly by adding probiotic to the patients. However, in contrast to our results, in a study performed on severe traumatic brain-injured (TBI) patients, APACHE II and SOFA scores were not significantly affected by probiotic treatment.[40] These negative results might be due to the selected strain or dose of bacteria, or the type of patients studied (patients with severe TBI and Glasgow Coma Scale scores between 5 and 8).

Our results showed that there was not a significant difference in the levels of the biomarkers on the 4th day between two groups highlighting the fact that probiotics show their efficacy in the critically ill patients if used for the duration of more than 4 days. Therefore, it seems that the long-duration therapy might be the best method of probiotics administration in ICU.

Limitation of the study

Our study was a single-center study with 40 patients included. Therefore, future multi-center trials with larger sample sizes of critically ill patients are required to achieve more solid results. In addition, our study was performed on surgical patients; hence, for routine administration of probiotics in critically ill-patients, they should be examined in medical or mixed type ICUs. Furthermore, further trials are needed to define the best dosage and optimal duration of therapy in these patients.

CONCLUSION

The results of this trial are encouraging and suggest that administration of probiotics in critically ill patients is associated with clinical benefits in relation to matched placebo-treated patients: They significantly reduce the levels of inflammatory biomarkers as well as APACHE and SOFA scores. Furthermore, significant increase in protein C levels could be detected. Therefore, probiotics could reduce inflammation in critically ill-patients and might be considered as an adjunctive therapy in the treatment of critically ill-patients. However, further studies with larger sample size are required to clarify their usefulness in this group of patients.

AUTHORS' CONTRIBUTION

SS contributed in the conception of the work, conducting the study, collecting the samples, preparing the manuscript draft, approval of the final version of the manuscript, statistical analysis, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. ME contributed in the design of the work, statistical analysis, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. HH contributed in sample analyzing via commercially available enzyme-linked immunosorbent assay kit, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MM contributed in the design of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AM contributed in the conception of the work, conducting the study, collecting the samples, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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