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Effects of 4 weeks of *Lactobacillus plantarum* 299v supplementation on nutritional status, enteral nutrition tolerance, and quality of life in cancer patients receiving home enteral nutrition – a double-blind, randomized, and placebo-controlled trial

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Abstract. – OBJECTIVE: Several human trials have confirmed that *Lactobacillus plantarum* 299v (Lp299v) relief the gastrointestinal symptoms observed in patients with irritable bowel syndrome, such as nausea, vomiting, and diarrhea. These symptoms are similar to those associated with home enteral nutrition and they affect nutritional status as well as patients' quality of life. The aims of this study were to determine the effect of Lp299v on nutritional status, enteral formula tolerance, and quality of life in cancer patients.

PATIENTS AND METHODS: The current double-blind, randomized, and placebo-controlled study included 35 cancer patients receiving home enteral nutrition. There were 2 groups of participants consuming either 2 x 10^10 CFU of Lp299v (n=21) or placebo (n=14) for 4 weeks.

RESULTS: An increase in the serum albumin concentration was significantly higher in the Lp299v group than in the placebo group at the endpoint (p=0.032). Moreover, the changes in the frequency of vomiting and flatulence were significantly reduced at week 4 compared to baseline in the Lp299v group (p=0.0117). The improvement of quality of life was observed in both groups; however, with no statistically significant differences between the analyzed groups (p>0.05).

CONCLUSIONS: We have demonstrated that administration of Lp299v in cancer patients receiving home enteral nutrition may improve laboratory parameters, predominantly the concentration of albumin, however, overall it does not have an impact on nutritional status. Lp299v may reduce the gastrointestinal symptoms related to enteral nutrition; notwithstanding, the improvement of quality of life may be the result of enteral nutrition rather than the effect of administration of Lp299v.

Key Words:
Lactobacillus plantarum 299v, Home enteral nutrition, Cancer, Nutritional status, Quality of life.

Abbreviations

QOL = quality of life; HEN = home enteral nutrition; EN = enteral nutrition; Lp299v = *Lactobacillus plantarum* 299v (DSM 9843); ITT = intention to treat; FAS = full set analysis; NRS 2002 = Nutritional Risk Screening 2002; TBW = total body water; BMI = body mass index; TLC = total lymphocyte count; GLIM = Global Leadership Initiative on Malnutrition; WHOQOL-BREF = World Health Organization Quality of Life-Bref; PEG = percutaneous endoscopic gastrostomy; ESPEN = European Society for Clinical Nutrition and Metabolism; FF = fiber-free nutrition formula; FE = fiber-enriched nutrition formula; FEP = fiber- and probiotic-enriched nutrition formula; CI = confidence interval; SGA = Subjective Global Assessment; MIE = Ivor Lewis minimally invasive esophagectomy; OE = open esophagectomy; FACT = Functional Assessment of Cancer Therapy.

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Introduction

Nutritional treatment is an important component of multidisciplinary anti-cancer therapy. According to many trials, appropriate nutritional support reduces the side effects of anti-cancer treatment, shortens the length of hospital stay, improves nutritional status and the clinical outcome as well as patients' quality of life (QOL)[1]. Home enteral nutrition (HEN) is recommended for patients with efficiently functioning gastrointestinal tract who do not require hospitalization[1,2]. According to Villar Taibo et al[3], as many as 75% of patients qualified for HEN are malnourished. Furthermore, several trials showed that HEN positively affects the patients' nutritional status presenting multiple benefits; however, enteral nutrition (EN) may also be associated with some complications, such as nausea, vomiting, flatulence, as well as abdominal pain[4]. Notwithstanding, diarrhea is the most prevalent side effect during HEN. It has an impact on overall recovery in the postoperative period, causes fluid and electrolyte loss, prolongs the length of hospital stay, as well as increases mortality and morbidity[4]. The incidence of diarrhea in patients receiving EN varies between 12 and 68% patients[5,6]. However, the pathogenesis of this symptom involves several factors. It is mainly caused by the unadjusted speed of the enteral formula administration and gastrointestinal tract's reaction to substances with higher osmolarity. In cancer patients, diarrhea can be an adverse event during anti-cancer therapy (e.g., chemotherapy-related or radiotherapy-related diarrhea). Another hypothesis is that diarrhea might be caused by changes in the gut microbiota[4,7]. Overall, the inadequate composition of gut microbiota has been linked to poor eating habits (high-fat diet, low fiber intake), administration of antibiotics, side effects of anti-cancer therapy, and surgical procedures[8-11]. In cancer patients, the increased abundance of several specific bacteria was noted. For instance, the high count of *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Streptococcus bovis*, *Peptostreptococcus anaerobius*, *Enterococcus faecalis*, as well as *Helicobacter pylori* is observed. These bacteria have been described as colorectal cancer-associated pathogens[7,10]. Moreover, the low level of *Bifidobacteria* and high level of *Clostridia* in patients who experienced diarrhea during EN were noted[4,12]. It should be emphasized that *Clostridia* is known as pathogenic bacteria causing diarrhea[4].

Currently, the administration of probiotics is one of the methods used to modify gut microbiota[13,14]. *Lactobacillus plantarum* 299v DSM 9843 (Lp299v) is a probiotic strain belonging to the Firmicutes and Gram-positive lactic acid bacteria[15,16]. It is commonly found in diet and is also able to reside in human colonic mucosa *in vivo* due to a specific mechanism of mannose adhesion[17]. Lp299v demonstrates high tolerance to acidic environments in gastric, as well as alkaline in the duodenum[18]. It has immunomodulatory properties increasing the anti-inflammatory IL-10 synthesis and secretion. Moreover, it increases the transcription MUC2 and MUC3, thus secretion of mucins being glycoproteins, which provide protection for the intestinal mucosal surfaces[19]. Lp299v has antibacterial activity against pathogens, such as *Listeria monocytogenes*, *Escherichia coli*, *Enterococcus faecalis*, as well as *Clostridium difficile*[18]. Kujawa-Szewieczek et al[15] trial has confirmed that routine use of Lp299v may prevent *Clostridium difficile* infection during antibiotic therapy in nephrology and transplantation ward; these results were also confirmed in one-year extended study[20]. Lp299v is also recommended for patients suffering from irritable bowel syndrome (IBS). Its administration has been found to relieve symptoms, such as relapsing abdominal pain, flatulence, and diarrhea[21].

As it was mentioned above, gastrointestinal symptoms occurring during EN are similar to those in IBS. The data regarding EN in combination with probiotics are limited; moreover, there is no data assessing the role of Lp299v in cancer patients receiving HEN. The primary aim of this randomized, double-blind, and placebo-controlled study was to determine the effect of the Lp299v on the nutritional status of cancer patients receiving HEN. The secondary aims were to assess the role of Lp299v in the improvement of EN tolerance and patients' QOL.

Patients and Methods

Patients

Participants (n=35) were recruited by surgeon and nutritionist in Nutritional Counselling Centre Copernicus in Gdansk and Department of Clinical Nutrition and Dietetics (Medical University of Gdansk), Poland. Inclusion criteria were age ≥18 yr., the presence of cancer, artificial access to the alimentary tract (naso-gastric tube, gastrostomy, percutaneous endoscopic gastrostomy, je-

junostomy, micro jejunostomy), qualification for HEN, written consent to take part in the study. Exclusion criteria included: age <18 yr., patients requiring home parenteral nutrition, not being able to attend the visit in the study center.

Study Design

This study was designed as a randomized, double-blind, and placebo-controlled to assess the efficiency of Lp299v in cancer patients receiving HEN. Treatment duration was 4 weeks with 1 visit after completing the 4th week. The study protocol has been approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdansk (identifier: 422/2016). All participants gave written informed consent prior to participation in this study and the information about this trial was explained to them. This study has been registered in ClinicalTrials.gov (identifier: NCT03940768).

The flow chart of this study is presented in Figure 1. A total of 35 participants were randomized into two groups: one group receiving probiotic – Lp299v (n=21) and the control group receiving placebo (n=14). Among these 35 participants, 10 were excluded, because they did not complete the 4 weeks treatment due to resignation or death.

Study Products

The study test product was Sanprobi IBS® containing 10^{10} CFU of Lp299v (Sanprobi IBS® Sanprobi Sp. z o.o., Sp. k., Szczecin, Poland; producer of powder – Institute Rosell-Lallemand, Montreal, Canada; LP299v owner of probiotic strain – Probi AB, Lund, Sweden). Placebo was produced and packed by the same company and did not contain any microorganisms. Placebo capsule weighed 410 mg +/- 7.5% and contained potato starch – 403 mg and magnesium stearate (magnesium salts of fatty acids) – 7 mg. Both the test and placebo products had the same appearance, structure, and taste. The study products were stored at refrigerator temperature. The participants were instructed to take one capsule of probiotic/placebo product in the morning after breakfast and one capsule in the evening after dinner for 4 weeks. The patients who could not swallow were provided by mixed capsules' powder with 20 ml of water or saline (in the case of patients with jejunostomy and micro jejunostomy). All participants were given standard normo-caloric enteral formulas with no additional fiber (average dose – 1500 ml) during 4-week therapy. The provision of enteral formula was adjusted to calorie requirements calculated with the Harris-Benedict Equation. The advised speed of administration was: (a) jejunostomy – 30-40 ml/h during first 4-7 days than reaching maximum tolerable dose/h (maximum 120 ml/h); (b) gastric access – repeated – 200-300 ml bolus followed by a 2-3 h break.

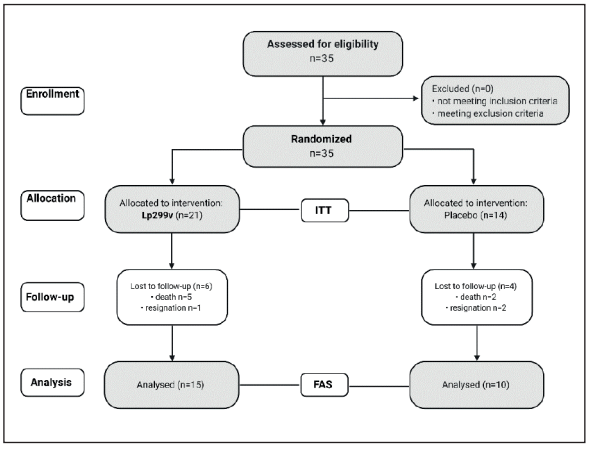

Figure 1. A flow-chart of the study design. ITT – intention to treat; FAS – full set analysis.

Sample Size and Randomization

After meeting the inclusion criteria and obtaining consent agreement, participants were randomized to receive a probiotic or placebo product. The randomization ratio was 1:1 and was performed by means of researcherandomizer.com software, typically used by clinical research associates. The researches and participants were blinded to the treatment arm.

When computing a priori sample size, we anticipated that the probiotic intervention will decrease the weight loss by 10% with an SD of around 15% for weight change. Calculating the mean weight loss in a 70 kg men and assuming 1:1 allocation ratio and 80% statistical power we evaluated that the number of participants will be 36. We randomly allocated 35 participants to receive either probiotic product or placebo. The required sample size was evaluated using the G-power analysis software.

Outcomes

Primary Outcomes

The primary outcome was the improvement of nutritional status in a probiotic-receiving group in comparison to placebo-receiving pa-

tients. The nutritional status was evaluated by means of anthropometric and laboratory parameters, as well as the Nutritional Risk Screening 2002 tool (NRS 2002 tool). Anthropometric parameters were: the percentage of unintentional weight loss during last the 6 months before the intervention, the composition of body mass (fat mass, muscle mass, total body water – TBW) and Body Mass Index (BMI). The body mass analysis was performed using BIA analyser – *Medical Jawon* in Department of Clinical Nutrition and Dietetics, Medical University of Gdansk, Poland. Laboratory tests included the serum concentration of albumin, total protein, and total lymphocyte count (TLC). The blood samples were taken in Nutritional Counselling Centre Copernicus in Gdansk and next given to the laboratory to conduct the analysis The anthropometric parameters and laboratory analyses were collected at baseline and after 4 weeks. The anthropometric parameters were noted at baseline and after 4 weeks.

Moreover, by means of NRS 2002 we evaluated the nutritional status of study participants but only at baseline. Malnutrition was diagnosed and categorized according to the Global Leadership Initiative on Malnutrition (GLIM) criteria (Stage 1 – moderate malnutrition, Stage 2 – severe malnutrition). The severity grading is based on phenotypic criteria. Stage 1 requires one of the following criteria: weight loss (5-10% within the past 6 months, or 10-% beyond 6 months), low BMI (<20 kg/m² if < 70 yr., <22 kg/m² if ≥ 70 yr.), and reduced muscle mass (mild to moderate deficit). Stage 2 – one of the following phenotypic criteria must be met: weight loss (>10% within the past 6 months or >20% beyond 6 months), low BMI (<18.5 kg/m² if < 70 yr., <20 kg/m² if ≥ 70 yr.), and reduced muscle mass (severe deficit).

Secondary Outcomes

The secondary outcomes were the improvement of EN tolerance and patients' quality of life. The tolerance of EN was assessed with authors own questionnaire referring to number of stools, frequency of vomiting and flatulence. The questionnaire was filled every day by patients and analyzed during the follow-up visit after 4 weeks. In order to assess patients' quality of life, we used the World Health Organization Quality of Life-BREF (WHOQOL-BREF) questionnaire. It contains 26 questions divided into 4 domains (D1 – environmental, D2 – psychological, D3 – somatic, and D4 – social factors); moreover, first question (Q1 – How would you rate your quality of life?") regards patients' self-assessment of QOL (where 1 point means "very poor" and 5 "very good") and second (Q2 – "How satisfied are you with your health?") patients' self-assessment of status of health (where 1 point means "very dissatisfied" and 5 "very satisfied"). The QOL was checked at baseline and after 4 weeks. The more points, the better QOL.

Statistical Analysis

The statistical analyses have been performed using the statistical suite StatSoft Inc. 2014 STATISTICA version 12.0. www.statsoft.com and Microsoft Excel. The quantitative variables were characterized by the arithmetic mean of standard deviation or median or max/min (range) and 95% confidence interval. The qualitative variables were presented with the use of count and percentage. In order to check if a quantitative variable derives from a population of normal distribution, the Shapiro-Wilk test has been used. Whereas to prove the hypotheses on homogeneity of variances Leven (Brown-Forsythe) test has been utilized. Statistical significance of differences between two groups (unpaired variables model) was processed with the *t*-Student's test (or Welch test in the case of lack of homogeneity) or U Mann-Whitney test (in cases where conditions of performing the *t*-Student's test were not satisfied or for variables measured by ordinal scale). The significance of difference between more than two groups were assessed with F-test (ANOVA) or Kruskal-Wallis (if AVOVA conditions were not fulfilled). In the case of statistically significant differences between two groups post hoc tests were utilized (Tukey test for F or Dunn for Kruskal-Wallis). In the case of two paired variables, *t*-Student or Wilcoxon signed-rank (if *t*-Student conditions are not fulfilled or for variables measured in ordinal scale) test was utilized. The significance of difference between more than two variables in the paired variables model has been checked by analysis of variance with repeated measurements or by Friedman test (if analysis of variance conditions are not satisfied or for variables measured in ordinal scale). Chi-squared tests for independence were used for qualitative variables (with the use of Yates correction for cell counts below 10, with check of Cochrane's conditions or with Fisher's exact test respectively). In order to determine dependence, strength and direction between variables,

correlation analysis was used by determining the Pearson or Spearman's correlation coefficients. In all the calculations the statistical significance level of p=0.05 has been used.

Results

Patients' Baseline Characteristics

A total of 35 participants were included in this trial (Table I). Among these, 10 were excluded due to resignation or death. 82.85% of ITT patients and 80% of FAS were severe malnourished according to GLIM criteria before intervention. No significant differences were observed between the Lp299v groups and the placebo group in terms of age, gender, type of cancer, artificial access to the alimentary tract, NRS 2002, and percent of unintentional weight loss during last 6 months at baseline.

Laboratory Parameters

The mean changes within the laboratory parameters over 4 week period in both groups are shown in Figure 2. The increase of the level of albumin in blood serum was significantly higher in Lp299v group than in the placebo group after week 4 (baseline 41.7±3 *vs.* 39.7±6.2; after treatment 43.2±4.3 *vs.* 38±7.1; p=0.032; respectively). In Lp299v group the concentration of albumin in blood serum was significantly increased after week 4 in compared to baseline (p=0.0335); but not in placebo group (p=0.594). Overall increase of total protein after 4 week in comparison with baseline was also observed in Lp299v group, however it was not statistically significant (70.6±3.7 *vs.* 71.4±4.7; p>0.05). This improvement was not present in placebo group (69.1±4 *vs.* 67.6±8.8; p>0.05). No statistically significant differences were found after treatment period in TLC/L in

Table I. Patients' characteristics ITT – Intention to treat; FAS – Full set analysis; PEG – percutaneous endoscopic gastrostomy; NRS 2002 – Nutritional Risk Screening; BMI – Body Mass Index.

	ITT Lp299v n = 21	FAS n = 15	ITT Placebo n = 14	FAS n = 10	ITT *p*-value	FAS
Age (yr.)	60 ± 10.9	60.9 ± 12.2	61.1 ± 8.9	62.2 ± 10.2	0.7566	0.7887
Gender (M/F)	16/5	10/5	11/3	8/2	0.8695	0.4670
Disease (%)					0.5992	0.6830
Gastric cancer	19	33.3	21.4	30		
Esophageal cancer	38.1	20	21.4	20		
Throat cancer	19	20	28.6	10		
Tongue cancer	4.8	6.7	14.3	20		
Tonsil cancer	9.5	0	0	0		
Gum cancer	4.8	6.7	0	0		
Sinus cancer	4.8	6.7	0	0		
Lung cancer	0	6.7	7.1	10		
Pancreatic cancer	0	0	7.1	10		
Craniofacial cancer	0	0	7.1	0		
(%)					0.2265	0.1619
PEG	47.6	53.3	42.9	40		
Jejunostomy	47.6	46.7	28.6	30		
Micro jejunostomy	0	0	7.1	10		
Naso-gastric tube	4.8	0	21.4	20		
(%)					0.9043	0.8183
NRS 2002 tool score < 3	0	0	0	0		
3	4.8	6.7	7.1	0		
4	19	20	21.4	30		
5	61.9	53.3	50	50		
6	14.3	20	21.4	20		
BMI (kg/m²)	21.2 ± 3.4	22 ± 3.1	21.4 ± 3.9	21.5±3.6	0.8916	0.7083
% of unintentional weight loss (last 6 months)	19.3 ± 9.9	17.1 ± 8.6	14.7 ± 6.4	13.9±7.0	0.1305	0.3356
Albumin (g/L)	40.7 ± 4.2	41.7 ± 3	39.7 ± 5.4	39.7 ± 6.2	0.5516	0.3571
Total protein (g/L)	69.9 ± 4.2	70.6 ± 3.7	68.6 ± 5.0	69.1 ± 4	0.4293	0.3362
TLC/ L	1620.2 ± 898.2	1628.5 ± 809.9	1286.7 ± 666.9	1454.4 ± 411.2	0.2440	0.5379

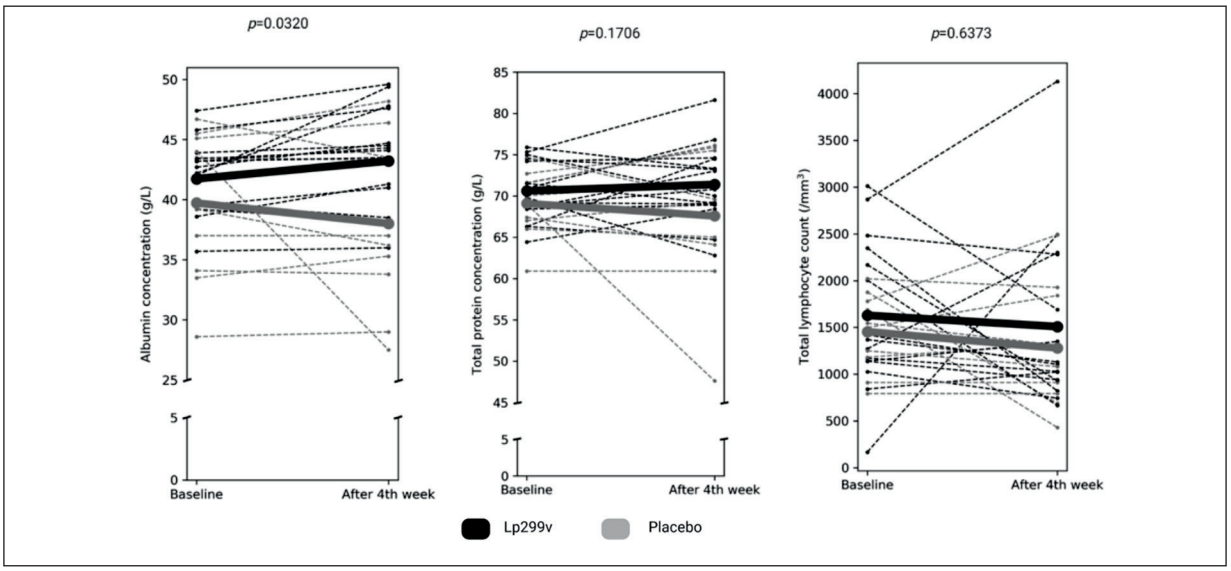

Figure 2. Changes in laboratory parameters in both groups. Dashed lines represent measurement of each patients before and after intervention. Solid lines represent mean values before and after intervention.

Lp299v and placebo group (1628.5±809.9 *vs.* 1454.4±411.2; 1508.6±938.3 *vs.* 1278.5±640.1; $p>0.05$; respectively).

Anthropometric Parameters

The alterations of anthropometric parameters are presented in Table II. There were no statistically significant changes in body mass, BMI, the content of fat mass, muscle mass, and TBW in both groups after 4-week treatment ($p>0.05$).

Tolerance of Enteral Nutrition

The frequencies of vomiting and flatulence (Figure 3) were significantly reduced at week 4 compared to baseline in Lp299v group (baseline: 1.5±2, after 4 week: 0.6±1.8, $p=0.0346$; baseline: 1.8±2.2, after 4 week: 0.5±1, $p=0.0117$; vomiting and flatulence respectively). However, it was not observed in placebo group (baseline: 1.9±1.9, after 4 week: 1.1±2.2, $p=0.1415$; baseline: 1±1.5, after 4 week: 0.1±0.3, $p=0.0679$, vomiting and flatulence respectively). The significant differences in frequency of vomiting and flatulence at baseline, at week 2, 3, and 4 between both groups were not observed ($p=0.5235$, $p=0.1741$, $p=0.5603$, $p=0.4054$; $p=0.4540$, $p=0.8244$, $p=0.6373$, $p=0.4540$, respectively).

The alterations of the stools frequency (Figure 3) per day were significantly reduced at week 4 compared to baseline in both groups (Lp299v baseline: 1.3±0.7, after 4 week: 0.8±0.4, $p=0.029$; placebo baseline: 1±0.4, after 4 week: 0.8±0.4, $p=0.0295$). No differences in changes of stools frequency at baseline, week 2, 3, and 4 between both groups were noted ($p=0.1548$, $p=0.3892$, $p=0.33$, $p=0.8852$, respectively).

Quality of Life

The alterations of quality of life are shown in Figure 4. The quality of life in Q1 was significant-

Table II. Changes in anthropometric parameters in both groups. TBW – total body water, BMI – Body Mass Index.

Anthropometric parameters	Lp299v		Placebo		*p*-value	
	Baseline	After 4 weeks	Baseline	After 4 weeks	Baseline	After 4 weeks
Body mass (kg)	65.7 ± 12.1	65 ± 10.8	64.5 ± 11.1	63.5 ± 11.8	0.8056	0.7461
BMI (kg/m²)	22.0 ± 3.1	21.7 ± 3.0	21.5 ± 3.6	21.1 ± 3.7	0.7083	0.6775
Fat mass (%)	14.2 ± 6.3	13.8 ± 6.3	13.6 ± 6.8	13.3 ± 6.6	0.8094	0.8352
Muscle mass (kg)	51.3 ± 8.7	50.6 ± 7.9	50.4 ± 7.6	49.7 ± 7.2	0.2673	0.2809
TBW (%)	36.8 ± 6.3	36.4 ± 5.7	36.3 ± 5.3	35.8 ± 4.9	0.8274	0.8019

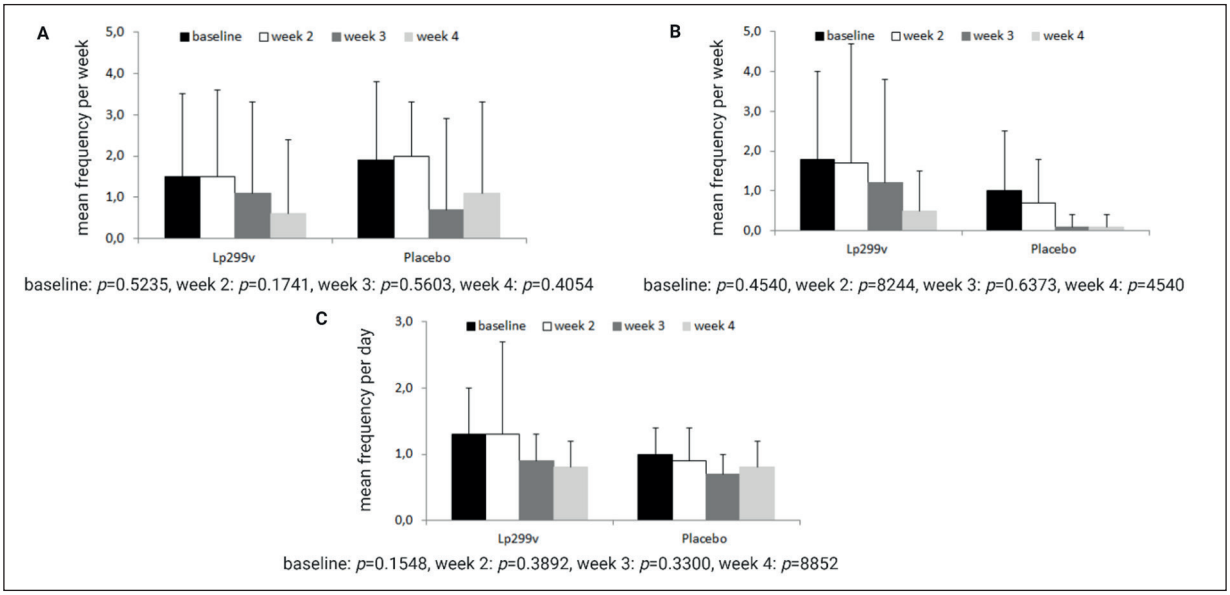

Figure 3. Changes in frequency of gastrointestinal symptoms and stools. A – vomiting; B – flatulence, C – stools. Lp299v – Lactobacillus plantarum 299v.

ly increased in Lp299v group after week 4 compared to baseline (2.7±1 *vs.* 3.3±0.8, p=0.0077). In Lp299v group the QOL was significantly higher in D2 and D4 after week 4 compared to baseline (12.7±2 *vs.* 13.5±2, p=0.028; 14.4±2.1 *vs.* 15.4±1.9, p=0.0414; respectively). In placebo group the increase of OQL was also observed in Q1, Q2, D2, D3, and D4, however, the differences were not significant compared to baseline. Moreover, no statistically significant differences between both groups in Q1, Q2, and domains were noted (p>0.05).

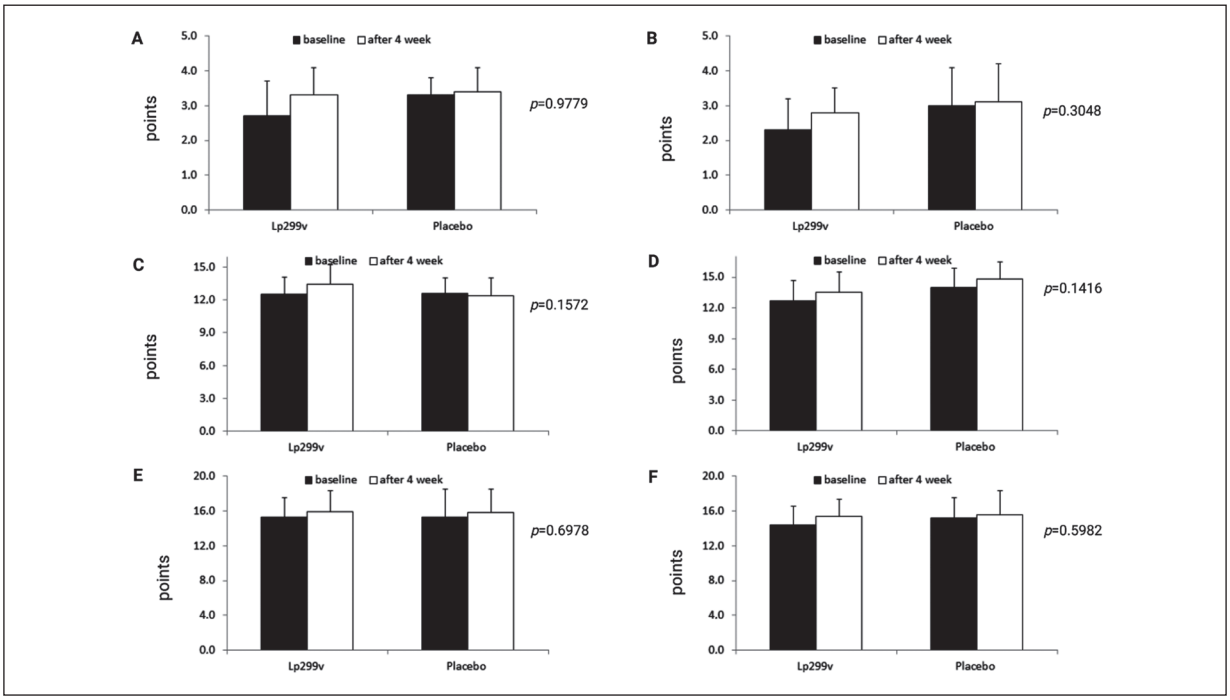

Figure 4. Changes in quality of life. A – Q1 (self-assessment of QOL); B – Q2 (self-assessment status of health); C – D1 (environmental); D – D2 (psychological); E – D3 (somatic); F – D4 (social factors). Lp299v – *Lactobacillus plantarum* 299v.

Discussion

The previous studies[4,22] have reported that adding probiotics to EN could improve immune function and decrease the incidence of diarrhea in cancer patients. Yi et al[23] conducted a meta-analysis which confirmed that early EN supplementation with probiotics effectively decreased the risk of infections (risk ratio [RR], 0.53; 95% confidence interval [CI], 0.44-0.65), mortality (RR, 0.56; 95% CI, 0.38-0.82), gastrointestinal complications (RR, 0.19; 95% CI, 0.13-0.25), and shortened an intensive care unit stay (mean difference [MD], −4.55; 96% CI, −5.91 to −3.19) in patients with severe head injury including also cancer patients. Notwithstanding, the effect of the Lp299v on nutritional status and the improvement of EN tolerance as well as the quality of life of cancer patients receiving HEN has not been studied earlier. Moreover, the present study treatment length is 4 weeks in comparison with other studies with shortened observation period. At the beginning, the administration of probiotic was planned for 12 weeks as it was previously registered in ClinicalTrials.gov before intervention. However, we decided to reduce the treatment period to 4 weeks, due to high mortality of cancer patients qualified for HEN.

Probiotics can reduce gastrointestinal symptoms, such as nausea, bloating, and diarrhea[21,24]. Zhao et al[4] showed that a combination of fiber and probiotics with EN significantly reduced the occurrence of diarrhea associated with EN in postoperative patients with gastric cancer. The incidence of diarrhea during 7 days treatment was 60% in patients of FF group (fiber-free nutrition formula), 30% of FE (fiber-enriched nutrition formula), and 5% of FEP (fiber- and probiotic-enriched nutrition formula). The similar results were obtained by Xie et al[22] confirming that diarrhea caused by EN occurred less frequently in the gastric cancer patients receiving probiotics compared to controls. Importantly, studies have shown that probiotics efficacy is strain-specific[25]. Lönnermark et al[26] in a double-blind, placebo-controlled trial investigated the effect of Lp299v (in a dose 10^{10} CFU per day) on the incidence of antibiotic-associated gastrointestinal symptoms. The significant reduction of the frequency of loose stools (odds ratio (OR), 0.69; 95% confidence interval (CI), 0.52-0.92; p=0.012) and nausea (OR 0.51; 95% CI, 0.30-0.85; p=0.0097) after administration of Lp299v was noted[26]. In the current study, the frequency of vomiting, flatulence, and stools were significantly reduced at week 4 compared to baseline in Lp299v group (p=0.0346, p=0.0117, p=0.024, respectively). However, the significant differences between Lp299v and placebo group were not observed. Moreover, the frequency of flatulence was reduced week-by-week in Lp299v group and the number of stools started to decline in week 3 and 4, which was not noted in the placebo group. It may be associated with the modification of gut microbiota after administration of Lp299v. To sum up, the administration of Lp299v potentially may reduce the gastrointestinal symptoms in patients receiving HEN.

According to the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines, all cancer patients should be screened regularly for the risk of malnutrition[27]. In the above mentioned study by Zhao et al[4], the assessment of nutritional status in patients with gastric cancer (n=120) receiving EN for 7 days was based on BMI (FF group 21.41±2.20 kg/m², FE 21.73±2.65, FEP 21.83±3.12; p=0.89) and laboratory parameters (the level of albumin – FF 37.71±2.72 g/L, FE 37.01±2.73, FEP 36.30±3.28, p=0.34; prealbumin – FF 192±6.72 mg/L FE 188±8.41, FEP 188±7.43, p=0.16; transferrin – FF 1.83±0.27 mg/L, FE 1.70±0.31, FEP 1.85±0.35, p=0.27; total lymphocyte count – FF 1.25±0.42 x 10^9/L, FE 1.15±0.34, FEP 1.13±0.32, p=0.53). The statistically significant difference between 3 groups in terms of these laboratory parameters after 7 days of EN was not observed[4]. Similarly, in Xie et al[22] study including patients with gastric cancer (n=140) receiving EN in combination with probiotics or placebo for 8 days, no difference was found between two groups after the treatment period. In the current study, it was confirmed that Lp299v significantly increased the level of albumin in the blood serum in comparison with the placebo group (p=0.032). However, the significant increase of the level of total protein was not observed (p>0.05). Similarly, no significant changes in TLC were observed. Since, the half-life of albumin is around 21 days, it is not accurate as a laboratory parameter to determine short term alterations. This may be the reason why in the current study the changes of this parameter were observed on the contrary to the above mentioned studies (4 weeks *vs.* 7 and 8 days). The concentration of albumin – being a negative acute phase protein – is decreased not only in malnutrition but also in the presence of inflammation[28]. Therefore, the increase in the albumin level after administration of Lp299v can suggest improvement in

the nutritional status and/or the reduction of inflammation. Due to non-significant improvement in anthropometric measures, increase in albumin concentration during Lp299v can be interpreted as anti-inflammatory effect of probiotic administration. However, other laboratory parameters describing the inflammation, such as C-reactive protein or pro- and anti-inflammatory cytokines were not included in this study, thus the immunomodulatory effect of Lp299v in cancer patients needs further studies. The lack of significant improvement in BMI, fat mass, muscle mass, and TBW after 4 week administration of Lp299v were observed.

GLIM initiative proposed severity grading of malnutrition into moderate and severe stages[29]. It has been published in 2019, therefore, data regarding clinical complications of those criteria and cancer are limited. In 2019, Contreras-Bolivar et al[30] presented results of observational and prospective study, which showed that SGA (Subjective Global Assessment) and GLIM criteria (mainly with hand grip strength) are useful in diagnosing malnutrition having also similar predictive value regarding six-month mortality in cancer inpatients. In the current study, 82.85% of ITT patients and 80% of FAS were severely malnourished according to the GLIM criteria before intervention. Therefore, it was almost impossible to improve their nutritional status.

The improvement of patients' quality of life is one of the most important goals of multi-disciplinary anti-cancer therapy. The effects of 3 months HEN on QOL and nutritional status after esophagectomy were assessed by Wu et al[31]. The participants were divided into 2 groups: undergoing Ivor Lewis minimally invasive esophagectomy with laparoscopic jejunal feeding tube placement (MIE group) and patients after open esophagectomy with naso-jejunal feeding tube placement (OE group). The results of this study indicated that patients who received HEN had a lower risk of malnutrition compared to patients who did not receive HEN (PG-SGA score, 5.7 *vs.* 7.9, $p<0.01$). The QLQ-30 questionnaire including physical, emotional, and cognitive functioning was used to evaluate the QOL. The mean scores of the global quality of life, physical function, role function, and social function were significantly higher in the MIE group compared to the OE group. It was concluded that after 3 months of HEN, patients in the MIE group had fewer symptoms and superior improvements in functioning in comparison to patients of the OE group[31]. Moreover, probiotics have been tested to improve cancer patients' quality of life. In double-blind, randomized, and placebo-controlled trial it was noted that administration of probiotics per 12 weeks improve cancer-related quality of life – Functional Assessment of Cancer Therapy (FACT) – (baseline *vs.* 12 weeks: 19.79 ± 4.66 *vs.* 21.18 ± 3.67, $p=0.04$) and fatigue-related FACT (baseline *vs.* 12 weeks: 43.00 (36.50-45.50) *vs.* 44.50 (38.50-49.00), $p=0.02$) in colorectal cancer survivors[32]. Ohigashi et al[33] also found that the administration of probiotics (containing *Bacillus* natto and *Lactobacillus acidophilus*) for 3 months was effective in the improvement of QOL after colorectal resection (n=77). However, none of the previous studies tested the efficacy of Lp299v in the cancer patient population. It was highly desirable since this strain was shown to be efficient in the reduction of gastrointestinal symptoms and as a consequence the improvement of QOL[34,35]. In the current study, the self-assessment of QOL was significantly increased after week 4 compared to baseline ($p=0.0077$) in patients receiving Lp299v; moreover, the significant improvement in the psychological domain and social factors was also noted ($p=0.028$; $p=0.0414$, respectively). In the placebo group, the improvement of QOL in Q1, Q2, psychological, somatic, and social factors were also showed. However, the differences were not significant compared to baseline. Furthermore, statistically significant differences between both groups were not observed ($p>0.05$); therefore, it is not certain if the improvement of QOL in Lp299v is associated with the administration of probiotic or it is the results of EN. This study only indicated that Lp299v was not inferior to placebo in changing QOL in cancer patients receiving HEN.

The present study has some limitations. The most important one is that this trial was conducted in a single center with a small sample size; moreover, the group was non-homogenous. We planned to recruit 40 participants as it was previously declared in ClinicalTrials.gov. However, eventually we recruited 35 patients and completed the current study in ClinicalTrials.gov regarding this number of participants. It is difficult and takes a lot of time to include a larger number of participants especially with the same type of cancer for 4 week treatment period, among others due to receive consent agreement and high mortality of cancer patients qualified for HEN. Therefore, there is a need to create a multi-center trial with a larger sample size. A noticeable fact

is that the majority of participants from the study were patients with advanced incurable cancer on palliative treatment. Improvement of nutritional status in this group is generally difficult or impossible to achieve. The main goal of nutritional treatment in this stage of the disease is to positively influence the quality of life.

Direction for Future Studies

This study showed possible directions for future investigations. Targeting a specific homogenous group of patients may bring more conclusive results. In our opinion individuals with early stages of cancer and good nutritional status/mild malnutrition may benefit more from probiotic intervention. Identification of microbiota changes will possibly enable researchers to determine the desired intervention method and study group.

Conclusions

In summary, in the current study we have demonstrated that administration of Lp299v in cancer patients receiving HEN may improve laboratory parameters mainly the concentration of albumin, however overall it does not have an impact on nutritional status. Lp299v may reduce the gastrointestinal symptoms related to EN; notwithstanding, the improvement of QOL may be the result of EN rather than the effect of administration of Lp299v.

Statement of Interest

Authors' declaration of personal interests: Karolina Skonieczna-Żydecka receives renumeration from probiotic company – Sanprobi (Sp. z o.o., Sp. k. Szczecin, Poland). The rest of the authors declared no conflict of interest.

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This study was funded; however, Sanprobi (Sp. z o.o., Sp. k. Szczecin, Poland) provided study products, i.e., probiotics and placebo.

Trial Registration

ClinicalTrials.gov (identifier: NCT03940768).

References

1) Gavazzi C, Colatruglio S, Valoriani F, Mazzaferro V, Sabbatini A, Biffi R, Mariani L, Miceli R. Impact of home enteral nutrition in malnourished patients with upper gastrointestinal cancer: a multicentre randomised clinical trial. Eur J Cancer Oxf Engl 2016; 64: 107-112.
2) Kaźmierczak-Siedlecka K, Folwarski M, Jankowska B, Spychalski P, Baran M, Szafrański W, Makarewicz W, Bryl E. Assessment of nutritional status of patients with cancer who are qualified for home enteral nutrition – a retrospective analysis. Eur J Transl Clin Med 2020; 3: 16-23.
3) Villar Taibo R, Martínez Olmos MÁ, Bellido Guerrero D, Vidal Casariego A, Peinó García R, Martís Sueiro A, González EC, Barreiro VR, Sánchez PC, Martínez RD, Iglesias MJR, Blanco BR, Valdés JR. Epidemiology of home enteral nutrition: an approximation to reality. Nutr Hosp 2018; 35: 511-518.
4) Zhao R, Wang Y, Huang Y, Cui Y, Xia L, Rao Z, Zhou Y, Wu X. Effects of fiber and probiotics on diarrhea associated with enteral nutrition in gastric cancer patients. Medicine 2017; 96: 8418.
5) Wiesen P, Van Gossum A, Preiser JC. Diarrhea in the critically ill. Curr Opin Crit Care 2006; 21: 149-154.
6) McNaught CE, Woodcock NP, Anderson ADG, MacFie J. A prospective randomised trial of probiotics in critically ill patients. Clin Nutr Edinb Scotl 2005; 24: 211-219.
7) Dai Z, Zhang J, Wu Q, Chen J, Liu J, Wang L, Xu J, Zhang H, Shi C, Li Z, Fang H, Lin C, Tang D, Wang D. The role of microbiota in the development of colorectal cancer. Int J Cancer 2019; 145: 2032-2041.
8) Garrett WS. Cancer and the microbiota. Science 2015; 348: 80-86.
9) Kaźmierczak-Siedlecka K, Dvořák A, Folwarski M, Daca A, Przewłócka K, Makarewicz W. Fungal gut microbiota dysbiosis and its role in colorectal, oral, and pancreatic carcinogenesis. Cancers 2020; 12: 1326.
10) Kaźmierczak-Siedlecka K, Daca A, Fic M, Van de Wetering T, Folwarski M, Makarewicz W. Therapeutic methods of gut microbiota modification in colorectal cancer management - fecal microbiota transplantation, prebiotics, probiotics, and synbiotics. Gut Microbes 2020; 11: 1518-1530.
11) Sun J, Chang EB. Exploring gut microbes in human health and disease: pushing the envelope. Genes Dis 2014; 1: 132-139.
12) Whelan K, Judd PA, Preedy VR, Simmering R, Jann A, Taylor MA. Fructooligosaccharides and fiber partially prevent the alterations in fecal microbiota and short-chain fatty acid concentrations caused by standard enteral formula in healthy humans. J Nutr 2005; 135: 1896-1902.
13) Raman M, Ambalam P, Kondepudi KK, Pithva S, Kothari C, Patel AT, Ravi Kiran Purama, Dave JM, Vyas BRM. Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. Gut Microbes 2013; 4: 181-192.
14) Chen D, Wu J, Jin D, Wang B, Cao H. Fecal microbiota transplantation in cancer management: Current status and perspectives. Int J Cancer 2019; 145: 2021-2031.

15) Kujawa-Szewieczek A, Adamczak M, Kwiecień K, Dudzicz S, Gazda M, WiĐcek A. The effect of Lactobacillus plantarum 299v on the incidence of Clostridium difficile infection in high risk patients treated with antibiotics. Nutrients 2015; 7: 10179-10188.

16) Rudzki L, Ostrowska L, Pawlak D, Małus A, Pawlak K, Waszkiewicz N, Szulc A. Probiotic Lactobacillus plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: a double-blind, randomized, placebo controlled study. Psychoneuroendocrinology 2019; 100: 213-222.

17) Johansson ML, Molin G, Jeppsson B, Nobaek S, Ahrné S, Bengmark S. Administration of different Lactobacillus strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. Appl Environ Microbiol 1993; 59: 15-20.

18) Goossens D, Jonkers D, Russel M, Thijs A, van den Bogaard A, Stobberingh E, Stockbrügger R. Survival of the probiotic, L. plantarum 299v and its effects on the faecal bacterial flora, with and without gastric acid inhibition. Dig Liver Dis 2005; 37: 44-50.

19) Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA. Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. Gut 2003; 52: 827-833.

20) Dudzicz S, Kujawa-Szewieczek A, Kwiecień K, Więcek A, Adamczak M. Lactobacillus plantarum 299v reduces the incidence of Clostridium difficile infection in nephrology and transplantation ward-results of one year extended study. Nutrients 2018; 10: 1574.

21) Ducrotté P, Sawant P, Jayanthi V. Clinical trial: Lactobacillus plantarum 299v (DSM 9843) improves symptoms of irritable bowel syndrome. World J Gastroenterol 2012; 18: 4012-4018.

22) Xie H, Lu Q, Wang H, Zhu X, Guan Z. Effects of probiotics combined with enteral nutrition on immune function and inflammatory response in postoperative patients with gastric cancer. J BUON 2018; 23: 678-683.

23) Yi LJ, Tian X, Shi B, Pi YP, Chen WQ. Early enteral nutrition supplemented with probiotics improved the clinical outcomes in severe head injury. Medicine 2019; 98: 15426.

24) Wei D, Heus P, van de Wetering FT, van Tienhoven G, Verleye L, Scholten RJ. Probiotics for the prevention or treatment of chemotherapy- or radiotherapy-related diarrhea in people with cancer. Cochrane Database Syst Rev 2018; 31: 008831.

25) McFarland LV, Evans CT, Goldstein EJC. Strain-specificity and disease-specificity of probiotic efficacy: a aystematic review and meta-analysis. Front Med 2018; 5: 124.

26) Lönnermark E, Friman V, Lappas G, Sandberg T, Berggren A, Adlerberth I. Intake of Lactobacillus plantarum reduces certain gastrointestinal symptoms during treatment with antibiotics. J Clin Gastroenterol 2010; 44: 106-112.

27) Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, Hütterer E, Isenring E, Kaasa S, Krznaric Z, Laird B, Larsson M, Laviano A, Mühlebach S, Muscaritoli M, Oldervoll L, Ravasco P, Solheim T, Strasser F, de van der Schueren M, Preiser J. ESPEN guidelines on nutrition in cancer patients. Clin Nutr 2017; 36: 11-48.

28) Gulhar R, Jialal I. Physiology, Acute Phase Reactants. In: Stat Pearls Publishing; 2019.

29) Cederholm T, Jensen GL, Correia MITD, Gonzalez MC, Fukushima R, Higashiguchi T, Baptista G, Barazzoni R, Blaauw R, Coats A, Crivelli A, Evans DC, Gramlich L, Fuchs-Tarlovsky V, Keller H, Llido L, Malone A, Mogensen KM, Morley JE, Muscaritoli M, Nyulasi I, Pirlich M, Pisprasert V, de van der Schueren MAE, Siltharm S, Singer P, Tappenden K, Velasco N, Waitzberg D, Yamwong P, Yu J, Van Gossum A, Compher C. GLIM criteria for the diagnosis of malnutrition – a consensus report from the global clinical nutrition community. Clin Nutr 2019; 38: 1-9.

30) Contreras-Bolívar V, Sánchez-Torralvo FJ, Ruiz-Vico M, González-Almendros I, Barrios M, Padín S, Alba E, Olveira G. GLIM criteria using hand grip strength adequately predict six-month mortality in cancer inpatients. Nutrients 2019; 11: 2043.

31) Wu Z, Wu M, Wang Q, Zhan T, Wang L, Pan S, Chen G. Home enteral nutrition after minimally invasive esophagectomy can improve quality of life and reduce the risk of malnutrition. Asia Pac J Clin Nutr 2018; 27: 129-136.

32) Lee JY, Chu SH, Jeon JY, Lee MK, Park JH, Lee DC, Lee JW, Kim NK. Effects of 12 weeks of probiotic supplementation on quality of life in colorectal cancer survivors: a double-blind, randomized, placebo-controlled trial. Dig Liver Dis 2014, 46: 1126-1132.

33) Ohigashi S, Hoshino Y, Ohde S, Onodera H. Functional outcome, quality of life, and efficacy of probiotics in postoperative patients with colorectal cancer. Surg Today 2011; 41: 1200-1206.

34) Nobaek S, Johansson ML, Molin G, Ahrné S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. Am J Gastroenterol 2000; 95: 1231-1238.

35) Niedzielin K, Kordecki H, Birkenfeld B. A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 2001; 13: 1143-1147.