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ORIGINAL RESEARCH

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Effect of a fermented dairy beverage on radiation-related gastrointestinal toxicity and inflammation in locally advanced cervical cancer patients, a randomized placebo-controlled pilot study

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Abstract

Background: Locally advanced cervical cancer (LACC) is a health burden in low- and middle-income countries. Treatment with chemoradiotherapy (CRT) causes intestinal inflammation and toxicity that affects the nutritional status and quality of life of patients. This study aimed to analyze the effect of a probiotic-rich fermented dairy beverage, compared to a placebo, on gastrointestinal toxicity, inflammatory response, chronic toxicity and quality of life in LACC patients. **Methods**: A randomized, double-blind, placebo-controlled pilot study assigned LACC patients to the probiotic group (n = 21) or the placebo group (n = 21). Intervention with probiotic or placebo beverages began two weeks before treatment and continued 90 days after treatment. The frequency and severity of toxicity symptoms, nutritional parameters, serum cytokines, fecal calprotectin, proctopathy, and quality of life were evaluated throughout treatment. **Results**: Lower frequency and severity of nausea and vomiting were observed in the probiotic group compared to placebo (15% *vs.* 40%). No differences among groups were observed in the frequency of other symptoms, including diarrhea. A trend was shown toward lower levels of inflammatory cytokines in the probiotic group. No significant differences were observed in the development of proctopathy. **Conclusions**: This study demonstrated the fermented dairy beverage's beneficial effect on reducing the frequency and severity of vomiting and a tendency to lower inflammation. Still, it did not provide benefits regarding other treatment-related toxicities, including diarrhea, probably due to the small sample size. **Clinical Trial Registration**: The Trial Registration Number is NCT05736315.

Keywords

Fermented dairy beverage; Probiotic; *Lactobacillus casei Shirota*; Gastrointestinal inflammation; Locally advanced cervical cancer; Chemoradiotherapy toxicity

1. Introduction

Cervical cancer (CC) is the fourth most common cancer and the fourth leading cause of cancer death in women worldwide [1]. For locally advanced CC (LACC), concomitant chemoradiation therapy (CRT) followed by brachytherapy (BT) is the elective treatment [2]. Since 2000, the superiority of combined treatment has been demonstrated in terms of disease-free periods and survival, decreasing pelvic and systemic recurrences [3]. However, great concern was raised during and after treatment because of CRT's acute and long-term toxicity. Patients had limited tolerance to telecobalt-60 irradiation, exhibiting an increased risk of disabling, severe, and in some instances, fatal gastrointestinal toxicity, documenting an incidence of grade 3 and 4 toxicity in the rectum [4]. Considering the high toxicity caused by radiotherapy (RT) with Cobalt-60, a variety of factors have been modified to reduce the morbidity of RT in LACC patients, including the use of linear accelerators for external beam radiation therapy (EBRT) at an irradiation dose of 50.4 Gy in 28 fractions [5].

Gastrointestinal toxicity occurs in 60%–80% of the patients during and after CRT. The most frequent symptoms include nausea, diarrhea, abdominal cramps, fecal urgency, anorexia, bleeding and reduction of intestinal motility, among others [6], and a higher risk of nutritional deterioration. Malnutrition and skeletal muscle loss are frequent in LACC patients during and

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after treatment. These conditions have been associated with higher tumor recurrence and lower overall survival (OS) [7, 8].

However, evidence suggests that the gut microbiota may contribute to intestinal injury and toxicity related to CRT [9]. The diversity and abundance of gut microbiota species are remodeled in women treated with pelvic radiation therapy [10]. Also, bacterial metabolites and signaling molecules play a role in toxicity symptoms [9]. On the other hand, the diversity of gut microbiota has been positively correlated with better patient-reported gastrointestinal function during CRT [11]. Some mechanisms involve the immune system regulation through specific metabolites [9]. Probiotics may alter the microbiota composition and induce tolerance in the gut to promote health benefits [12–16]. Probiotics may confer protection during CRT because their metabolites induce mucus production in the gut mucosa and promote damaged tissue repair [17]. However, to our knowledge, there is still controversy regarding the specific probiotic species that may benefit patients who receive CRT [12, 18, 19]. Probiotics like *VSL#3*, *Lactobacillus casei DN-114 00*, *Lactobacillus acidophilus* + *Bifidobacterium*, *Lactobacillus acidophilus* or *Lactobacillus casei Shirota*, and Bifidobacterium breve have shown benefits reducing the incidence and severity of diarrhea, or the use of antidiarrheal medication [20–22]. However, these probiotic interventions are costly and inaccessible to low-income populations. Further, adequate probiotic species and doses have yet to be established.

Fermented foods have historically been part of the dietary habits of most cultures worldwide and have the advantage of being generally well-tolerated and cost-accessible to low-income populations [23]. Over the last decades, fermented foods have gained popularity for their reported benefits in gut health, cardiovascular health, metabolic health, mood improvement and immune regulation, among other health benefits [23, 24]. The influence of fermented foods on healthy subjects' microbiome and immune system was recently reported [25]. Following a 17-week dietary intervention with fermented foods, the participants had a decrease in many inflammatory markers and an increase in microbiota diversity [25], rendering this type of intervention promising to counter CRT treatment-related gastrointestinal toxicities and to regulate inflammation.

Among fermented foods, yogurt, fermented milk and fermented dairy beverages are the most frequently consumed and generally accepted in Western populations [26]. *Lactobacillus casei Shirota* is a Gram-positive anaerobic bacterium from the Firmicutes phylum commonly used in fermented dairy products with a high acceptance by the Mexican population. It is a probiotic that has been extensively studied for its health benefits. It regulates the immune system response in infection models and antitumor immunity, specifically through activating the Th1 response while modulating inflammation in healthy subjects [27–31]. It also regulates the gut microbiota and promotes intestinal integrity by activating resident macrophages [32, 33]. The use of probiotic *Lactobacillus casei Shirota* has also shown a promising improvement in stool consistency, reduction of diarrhea severity, and use of antidiarrheal medication in patients treated with pelvic radiotherapy [20, 34]. In other health conditions, *Lactobacillus casei Shirota*-based probiotic beverage has also prevented antibiotic-associated diarrhea [35]. *Lactobacillus casei Shirota*-based fermented dairy beverage also has the advantage of being resistant to gastric acid and surviving the gastrointestinal tract [36].

Thus, we hypothesized that a *Lactobacillus casei Shirota*-based fermented beverage could help regulate the inflammatory process and intestinal injury in LACC patients undergoing CRT, reducing treatment-associated gastrointestinal toxicity. We aimed to evaluate the effect of a fermented beverage with *Lactobacillus casei Shirota* on the frequency and severity of diarrhea and other gastrointestinal toxicities, systemic and local inflammation, and the development of radiation-induced proctopathy in LACC patients.

2. Materials and methods

2.1 Design

A randomized, double-blind, parallel placebo-controlled pilot study analyzing the effect of a fermented beverage with the probiotic *Lactobacillus casei Shirota* (Yakult) on gastrointestinal toxicity and inflammatory response in LACC patients undergoing CRT followed by BT at the Instituto Nacional de Cancerología (National Cancer Institute of Mexico).

2.2 Patients

Patients' eligibility criteria were 18 years and older, diagnosis with squamous cell carcinoma, adenosquamous, adenocarcinoma or glassy cell carcinoma, clinical stage IIB, and candidates for treatment with CRT followed by BT at the Instituto Nacional de Cancerología. Exclusion criteria were neuroendocrine histology, other primary tumors, previous treatment with RT or CRT, Karnofsky functional status below 80, and difficulty understanding the study's nature. The sample size for this pilot study was determined by convenience to establish the safety of probiotic consumption in LACC under CRT treatment. 42 LACC patients were recruited from 2006 to 2008, agreed to participate in the study, and signed an informed consent. Follow-up was carried out until 2019 to monitor patients and assess the development of proctopathy. Study protocol and data were collected at the Instituto Nacional de Cancerología.

Yakult© Mexico provided the Yakult© probiotic drink, as it is commercially manufactured, with 8×10^9 CFUs of *Lactobacillus casei Shirota* in a volume of 80 mL, and the placebo drink with the same organoleptic characteristics in the same volume but without the probiotic.

Yakult© Mexico carried out randomization through the sealed envelope system. Researchers and participants were both blinded to the group assigned. A third party not involved in the study was informed about the group to which each patient was assigned to take adequate measures in case of adverse events. Participants were randomly assigned 1:1 to one of two groups: (a) Fermented beverage group: patients consumed 80 mL of a fermented dairy beverage with 8×10^9 *Lactobacillus casei Shirota* (Yakult©); and (b) Placebo group: patients consumed 80 mL of a probiotic-free dairy beverage similar in color and flavor to the fermented drink. Nutritional characteristics of both drinks contained 55 kcal, 1

g of protein, 0.04 g of fat, 12.7 g of carbohydrates, 10.7 g of added sugars, 13 mg of sodium and 36 mg of calcium. Both groups consumed the beverage three times a day for 22 weeks, starting two weeks before treatment initiation and then three months after the conclusion of BT.

The patients received nutritional counseling and a diet individualized and tailored to their energy and protein requirements according to their baseline anthropometric measurements. Energy and protein requirements were calculated based on the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines on nutrition for cancer patients [37] as follows: E (kcal) = 30 kcal/kg of weight per day; Protein (g) = 1.5 g/kg of weight per day. The counseling consisted of restricting some foods known to exacerbate abdominal discomfort and of specific recommendations based on the patient's individual characteristics and access to food.

All patients were treated with CRT. Patients received 5–6 weekly cisplatin-based CT applications at 40 mg/m^2 doses. RT was administered concurrently, at a standard dose of 50 to 56 Gy of external beam radiation (Cobalt-60) in fractions of 2 Gy, five days a week for five weeks. After CRT, one or two applications of BT with Cesium-137 were given to reach a total dose to points A and B of at least 85 and 55 Gy, respectively.

2.3 Study procedures

Patients were evaluated at several time points throughout their treatment: (a) 2 weeks before starting CRT (wk -2); (b) starting CRT (t 0); (c) at the 3rd CRT cycle (wk 3); (d) at the 6th CRT cycle (wk 6); (e) after the first time of BT (BT 1); (f) after the second time of BT (BT 2); (g) 30 days after brachytherapy (30 d); (h) 60 days after brachytherapy (60 d); (i) 90 days after brachytherapy (90 d). After treatment completion, patients were followed up during periodic check-ins every 6 months for the first 3 years and then yearly for the rest of their lives. During these follow-up visits, the patients were primarily monitored for tumor recurrence and secondly for late-onset treatment-related toxicity; additionally, mortality was recorded.

Primary outcome measures included gastrointestinal toxicity, intestinal inflammation, and systemic inflammation. Secondary outcomes included nutritional status and quality of life. On each visit, anthropometric measurements (body weight, Body Mass Index, BMI), gastrointestinal toxicity (according to the Common Toxicity Criteria of Adverse Events, CTCAE v.3), blood tests, and quality of life (assessed using the European Organization for Research and Treatment of Cancer Quality of Life questionnaire (EORTC QLQ-C30 v.2)) were evaluated, and blood and fecal samples were obtained. Prognostic Nutritional Index (PNI) [(serum albumin g/dL $\times$ 10) + (0.005 $\times$ total lymphocyte count cells/μL)] and Neutrophil to Lymphocyte Ratio (NLR) were calculated.

To assess the systemic inflammatory response, we quantified serum cytokine levels on blood samples (Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interferon-γ (IFN-γ), Interleukin-17 (IL-17), Granulocyte Colony-Stimulating Factor (G-CSF), eotaxin, Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Monocyte Chemoattractant Protein (MCP-1), Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Interleukin-10 (IL-10), Vascular Endothelial Growth Factor (VEGF)). Calprotectin was quantified on fecal samples. As described elsewhere, serum cytokines and fecal calprotectin were determined by enzyme-linked immunosorbent assay (ELISA) [38]. ELISA reagent kits were purchased from Bio-Plex Multiplex (Bio-Rad, Hercules, CA, USA).

The instrument EORTC QLQ-C30 v.2 assessed the quality of life. To interpret the QLQ-C30 questionnaire, we considered the global health status [39].

2.4 Statistical analysis

Patient characteristics were described. The distribution of the quantitative independent variables was determined using Kolmogorov-Smirnov. The mean $\pm$ standard deviation was reported for the variables with normal distribution, and the median and interquartile range (IQR 25%–75%) for the variables with free distribution. Ordinal variables were reported as frequencies and percentages. Primary outcome measures were analyzed as follows. Toxicity was described as ordinal variables, compared through Fisher's exact test (when the expected count was <5) or chi-square for dichotomous variables. Levels of inflammation markers were compared in a bivariate analysis, using the Student's T test for quantitative variables with normal distribution and the Mann-Whitney U test for quantitative variables with free distribution. Secondary outcome measures were described. Depending on the distribution, the nutritional status variables and quality of life were analyzed using the Student's T or Mann-Whitney U tests. Data processing and analysis were performed using the statistical packages SPSS version 23 (IBM Corp., Armonk, NY, USA), and GraphPad Prism Version 9 (GraphPad Software, San Diego, CA, USA) was used to create graphics. Confidence intervals were built at 95%, and a value was declared statistically significant when $p < 0.05$.

3. Results

3.1 Clinical characteristics

A total of 42 women were analyzed; 21 patients (50%) were assigned to the experimental group that consumed a fermented dairy drink (probiotic group), and 21 patients (50%) were assigned to the placebo group that consumed a dairy drink with no probiotics (placebo group). All patients were analyzed per the protocol and for primary and secondary outcomes, according to the group assigned. No patients were lost to follow-up because all patients were at least 80% compliant with the intervention. No patients were excluded after randomization. The first patient in was recruited on 24 April 2005, and the last patient out was registered on 01 November 2019. Patients from the probiotic group were significantly older, with a mean of 56.3 $\pm$ 8.6 years, compared to the placebo group, with a mean of 50.32 $\pm$ 7.5 years (p = 0.02). More patients, 85.7% (n = 18) from the probiotic group, did not have any education compared to the placebo group (p = 0.04) (Table 1).

Squamous cell carcinoma was the most frequent histology in 95% of the probiotic group and 81% of the placebo group. There were no significant differences among groups regarding

TABLE 1. Clinical characteristics of CC patients.

Variables	Probiotics n (%)	Placebo n (%)	$p^{\dagger}$
	21 (50%)	21 (50%)	
Age (yr)*	56.30 ± 8.6	50.32 ± 7.5	**0.02**
Education			
No studies	18 (86)	12 (57)	**0.04**
Elementary school	3 (14)	5 (24)	0.43
Junior high school	0 (0)	4 (19)	0.10
Histology			
Adenocarcinoma	1 (5)	4 (19)	0.15
Squamous cell carcinoma	20 (95)	17 (81)	
Comorbidities			
None	13 (62)	14 (67)	
Obesity	7 (33)	7 (33)	0.31
T2DM and SAH	1 (5)	0 (0)	
Treatment			
CT (5 cycles)	1 (5)	2 (9)	
CT (6 cycles)	19 (90)	18 (86)	0.65
CT (7 cycles)	1 (5)	1 (5)	
RT dosage (Gy)[&]	50.4 (50.4–50.4)	50.4 (50.4–50.4)	0.70
No BT	0 (0)	1 (5)	
BT (1 application)	12 (57)	7 (33)	0.40
BT (2 applications)	9 (43)	13 (62)	

**Mean ± SD. [&]Median (IQR25-IQR75). [†]Chi-square and Fisher's exact test. yr: years; T2DM: Type 2 Diabetes Mellitus; SAH: Systemic Arterial Hypertension; CT: Chemotherapy; RT: Radiotherapy; BT: Brachytherapy; No BT: patients that did not receive BT. Bolded p-values indicate statistically significant differences ($p < 0.05$).*

comorbidities in both groups ($p = 0.31$). Most of the population (64%) did not present any comorbidity; seven patients (33%) in each group had obesity. Regarding treatment, there were no significant differences among groups (Table 1).

3.2 Toxicity symptoms developed during treatment are similar in patients from the probiotic and placebo groups

All patients tolerated the fermented and placebo drinks; no allergic reaction or intolerance to the products was reported. Fig. 1 depicts gastrointestinal toxicity symptoms comparing patients from the probiotics and placebo groups from the first CRT cycle up to 90 days after treatment completion. A minor frequency of patients developed anorexia with no differences among groups, except for week 5, where the probiotic group showed a significantly higher frequency (20% *vs.* 0%, $p = 0.048$) and a tendency to be more severe with up to 20% of patients with grades 1 and 2 during CRT (Fig. 1a). Nausea and vomiting are important toxicity symptoms presented with CRT. While patients from both groups had similar symptoms, on week 2, patients from the probiotic group had significantly less frequency of vomiting (29% *vs.* 62%, $p = 0.03$) compared to placebo. On weeks 3 and 6, patients in the probiotic group also tended to have less vomiting (29% *vs.* 55%, $p = 0.086$ and 15% *vs.* 40%, $p = 0.077$) compared to placebo. A trend towards more severe nausea and vomiting was observed in the placebo group, with up to 50% of patients with grade 2 nausea and up to 5% with grade 3 vomiting during CRT (Fig. 1b,c). Up to 50% of patients developed diarrhea during CRT; no significant differences in frequency and severity were found among groups. Both groups had similar frequency and severity of constipation. However, the probiotic group tended to have less constipation on week 5 (5% *vs.* 25%, $p = 0.093$) (Fig. 1e). Proctitis was similar in both groups during treatment; notably, the symptoms persisted after treatment in 25% of patients. Proctitis tended to be more severe in the probiotic group at 60 and 90 days after treatment completion, with 2% of patients presenting grade 3 proctitis (Fig. 1f). No differences were observed in mucositis, dehydration, dysphagia/esophagitis, stomatitis/pharyngitis, colitis or pancreatitis (Data not shown). No harm was reported by consumption of fermented dairy beverages or placebo beverages.

3.3 Nutritional status during treatment in patients from the probiotic and placebo groups

Because gastrointestinal toxicity symptoms impact food intake and nutrient absorption, we analyzed changes in nutritional

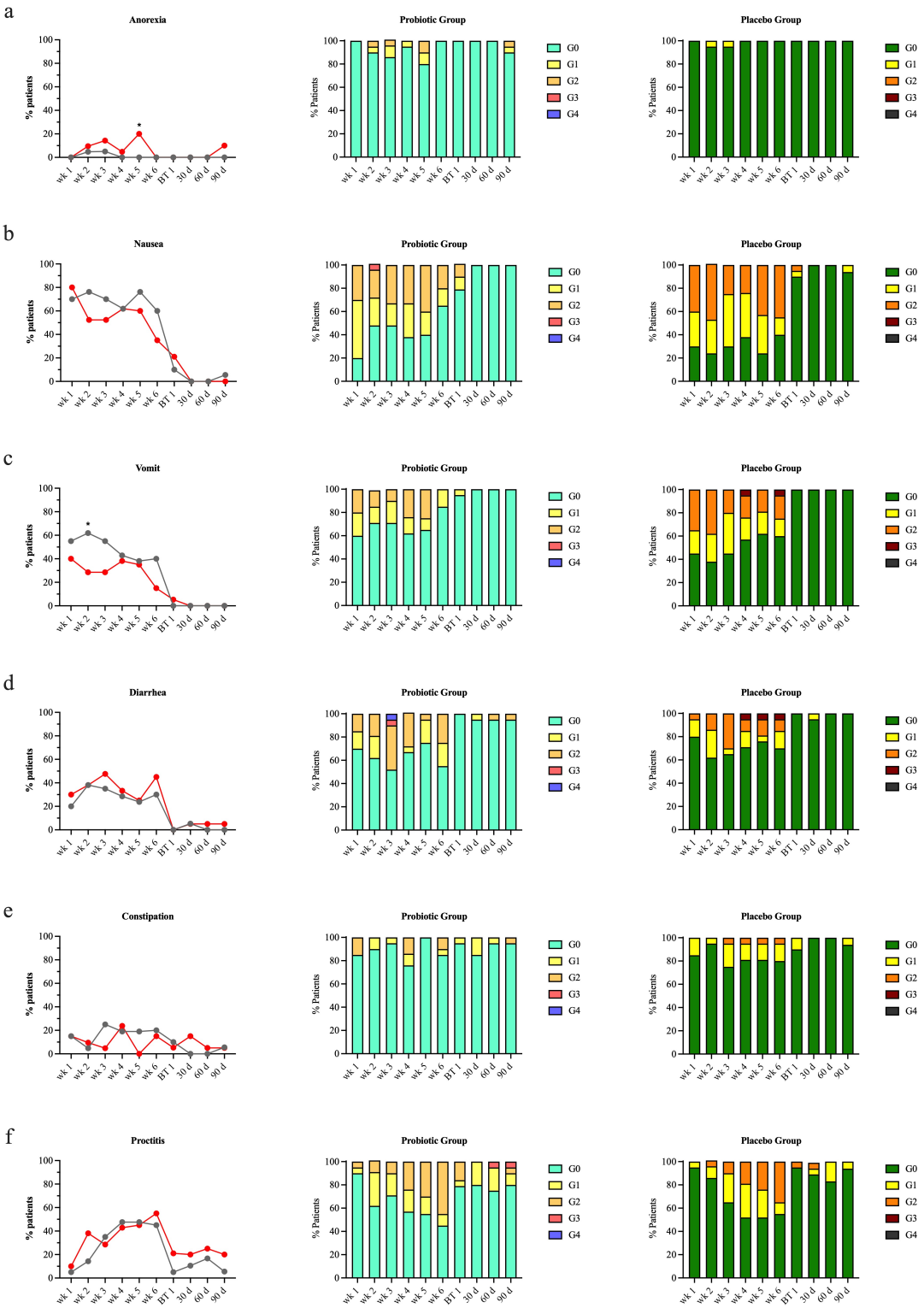

FIGURE 1. Gastrointestinal toxicity in LACC patients. Acute gastrointestinal toxicity developed during treatment in LACC patients from probiotic (n = 21) and placebo (n = 21) groups. (a) Anorexia. (b) Nausea. (c) Vomit. (d) Diarrhea. (e) Constipation. (f) Proctitis. Toxicity evaluation was performed after the first (wk 1), second (wk 2), third (wk 3), fourth (wk 4), fifth (wk 5) and sixth (wk 6) CRT cycles, after the first Brachytherapy application (BT 1), and at 30 days (30 d), 60 days (60 d) and 90 days (90 d) after treatment conclusion. The graph on the left depicts the percentage of patients with symptoms for the probiotic group (red line) and placebo group (gray line). The graph in the middle depicts the frequency of symptom severity in patients from the probiotic group, grades 0 through 4 (G0 = no symptom, CTCAE). The graph on the right depicts the frequency of symptom severity in patients from the placebo group, grades 0 through 4 (G0 = no symptom, CTCAE). *$p < 0.05$. BT: Brachytherapy.

parameters (Fig. 2). Two weeks before treatment began, patients from the probiotic group had a median BMI of 29.6 kg/m^2, compared to 27.8 kg/m^2 in the placebo group. No statistically significant differences were found among groups during treatment (Fig. 2a). However, for both groups of patients, a considerable weight loss (WL) was observed at the third cycle of CRT, with a median WL of 3% for the probiotic group and 4.7% for the placebo group ($p < 0.0001$). At 30 days after treatment completion, patients began to recover weight, with a median weight gain of 2.6% for the probiotic group and 4.1% for the placebo group (Fig. 2b). We compared the ponderal WL in both groups and found no difference (Fig. 2c,d).

To further assess the nutritional status of both groups of patients, we analyzed changes in the prognostic nutritional index (PNI). We observed no differences among groups, with a slight deterioration on the 3rd CRT cycle for both groups and a tendency towards a lower PNI in the probiotic group (Fig. 2e). The neutrophil/lymphocyte ratio (NLR), a marker of inflammation, climbed during treatment in both groups, with a maximum peak at the 3rd CRT cycle (wk 3) for the placebo group and at the 6th CRT cycle (wk 6) for the probiotic group; both groups recovered by day 30 after treatment completion (Fig. 2f).

3.4 Inflammatory response in patients from probiotic and placebo groups during treatment

It has been demonstrated that mucosal exposure to radiation causes tissue damage and the rapid expression of inflammatory cytokines, particularly IL-1β, IL-6, and those related to the acute response [40, 41]. Pelvic RT also induces dysbiosis, and altered microbiota may transfer tissue damage through the induction of inflammatory cytokines IL-1β, IL-6 and TNF-α [42]. An uncontrolled inflammatory acute response during treatment has been related to the development of pelvic radiation disease, and if it is not resolved to intestinal chronic inflammation and development of proctopathy [43, 44]. We analyzed multiple cytokines to evaluate if the intervention with the fermented beverage impacted the systemic inflammatory response (Fig. 3). Both groups' TNF-α, IL-1β and IL-6 expression seemed constant throughout treatment. TNF-α levels tended to be higher in the placebo group throughout treatment up to 60 days after treatment completion (Fig. 3a). A similar trend was observed with IL-1β, which was significantly higher in the placebo group since week -2 and during treatment at 6th CRT cycle ($p = 0.02$) (Fig. 3b). No differences were observed in the levels of IL-6 among groups (Fig. 3c). IL-2 behaved differently through treatment (Fig. 3d). IL-4 levels tended to be higher in the placebo group, with the highest expression observed before treatment began (Fig. 3e). In the probiotic group, IL-4 remained constantly low throughout the treatment. We observed no differences in the levels of IFN-γ among groups; a very high expression was detected throughout treatment (Fig. 3f). IL-17 tended to be constantly lower in the probiotic group than in placebo (Fig. 3g). The anti-inflammatory cytokine IL-10 remained constant in the probiotic group and tended to be higher before and during treatment in the placebo group (Fig. 3h).

Next, we analyzed levels of immune system-related growth and chemoattractant factors. G-CSF was significantly lower in the probiotic group before treatment (wk -2, $p = 0.02$; t 0, $p = 0.01$) and at the 6th CRT cycle (wk 6, $p = 0.02$) (Fig. 3i). The levels of GM-CSF tended to drop during treatment with CRT and then increase after BT treatment in the probiotic group. At the same time, no changes were observed in the placebo group (Fig. 3j). MCP-1 seemed lower throughout treatment in the probiotic group than placebo (Fig. 3k). No differences were observed in the eotaxin levels among groups. However, they tended to be constantly higher in the placebo group (Fig. 3l). VEGF expression remained low throughout treatment in the probiotic group (Fig. 3m) and tended to be higher in the placebo group, though no significant differences were observed. The highest expression was observed before treatment began and at the 1st BT application.

3.5 Acute intestinal inflammation and development of proctopathy in patients from the probiotic and placebo groups

We explored the local intestinal inflammatory response by quantifying fecal calprotectin (Fig. 4a). No significant differences were identified among the groups. The placebo group tended to secrete more calprotectin than the probiotic group by the 3rd CRT cycle (wk 3). At the 6th CRT cycle (wk 6), the probiotic group significantly increased calprotectin secretion ($p = 0.01$) and then significantly decreased by 30 days after treatment conclusion (Fig. 4a).

Fig. 4b shows the incidence of proctopathy in patients from probiotic and placebo groups. The median follow-up was 14.7 months (95% CI (Confidence Interval): 11.3–18.2). During this period, 14 patients in the probiotic group (66.7%) and 12 in the placebo group (57.1%) were diagnosed with proctopathy. Though a tendency towards increased proctopathy was observed in the probiotic group, no significant differences were observed in incidence, grade, or time to diagnosis ($p = 0.437$).

3.6 Quality of life

Fig. 5 shows the global health status scores of the QLQ-C30 questionnaire, measured over time in the probiotic group compared to the placebo group. Differences were observed among groups at week six and 1st BT application. A score closer to 100 was observed in the placebo group, being statistically significant ($p < 0.05$). In both groups, global health perception increases after treatment completion, improving quality of life.

4. Discussion

LACC continues to be a health burden, particularly in low- and middle-income countries such as Mexico [1]. CRT, followed by BT, remains the standard of treatment for these patients. Even though new targeted therapies are being studied, interventions to reduce treatment-related toxicity must be explored. Our study aimed to evaluate the effect of a fermented dairy beverage rich in probiotics on acute and chronic gastrointestinal toxicity and its impact on systemic and local inflammation.

Regarding gastrointestinal toxicity symptoms, we observed

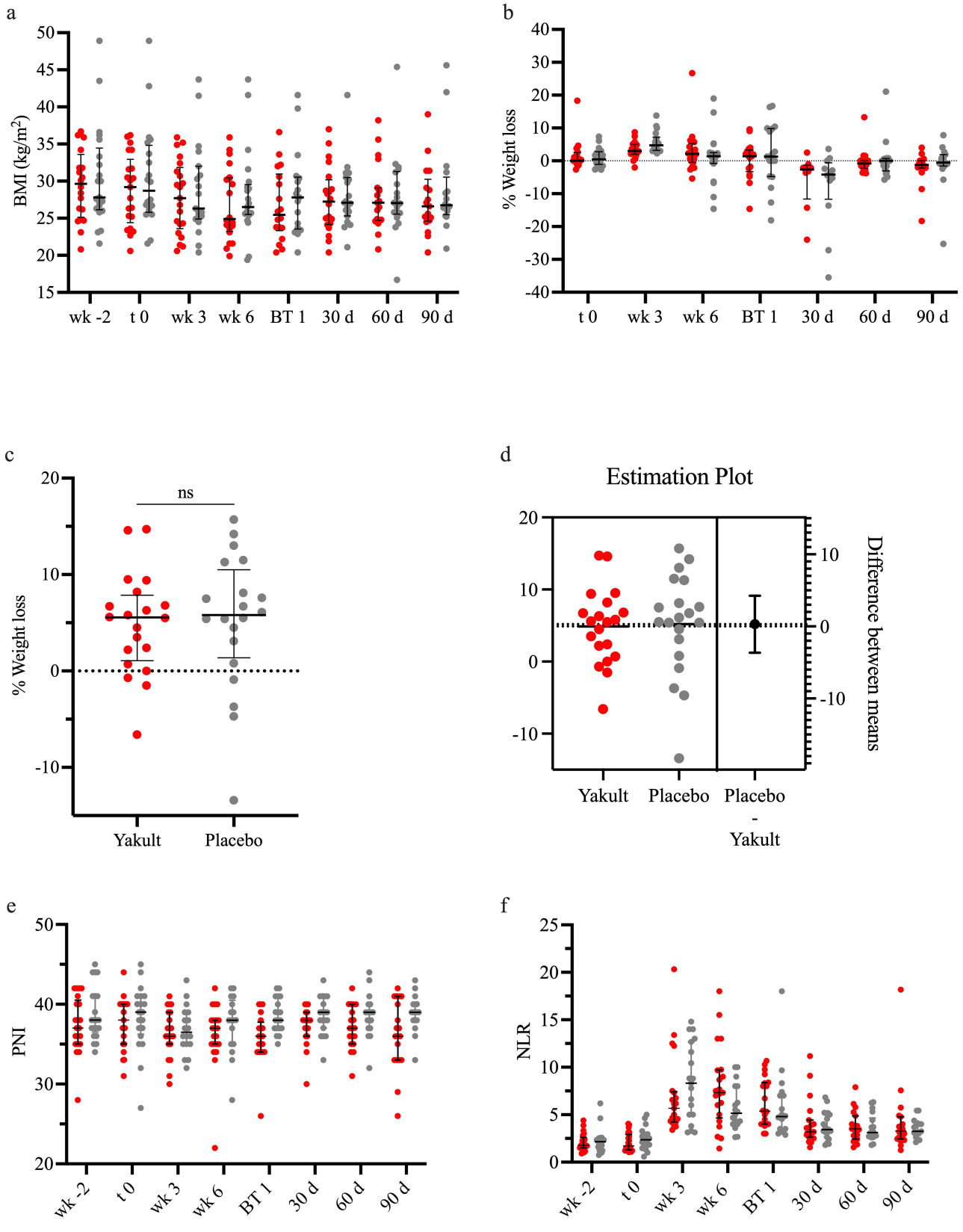

FIGURE 2. Nutritional status in LACC patients. Nutritional parameters during treatment in LACC patients from probiotic (n = 21) and placebo (n = 21) groups. Scatter dot plots of (a) BMI; (b) %Weight loss; (c) Ponderal %Weight Loss; (d) Weight Loss Difference between groups; (e) PNI; and (f) NLR. Probiotic group (red dots). Placebo group (gray dots). The line represents median ± IQR. BMI: Body Mass Index; PNI: Prognostic Nutritional Index; NLR: Neutrophil to Lymphocyte Ratio; BT: Brachytherapy; ns: Not statistically significant.

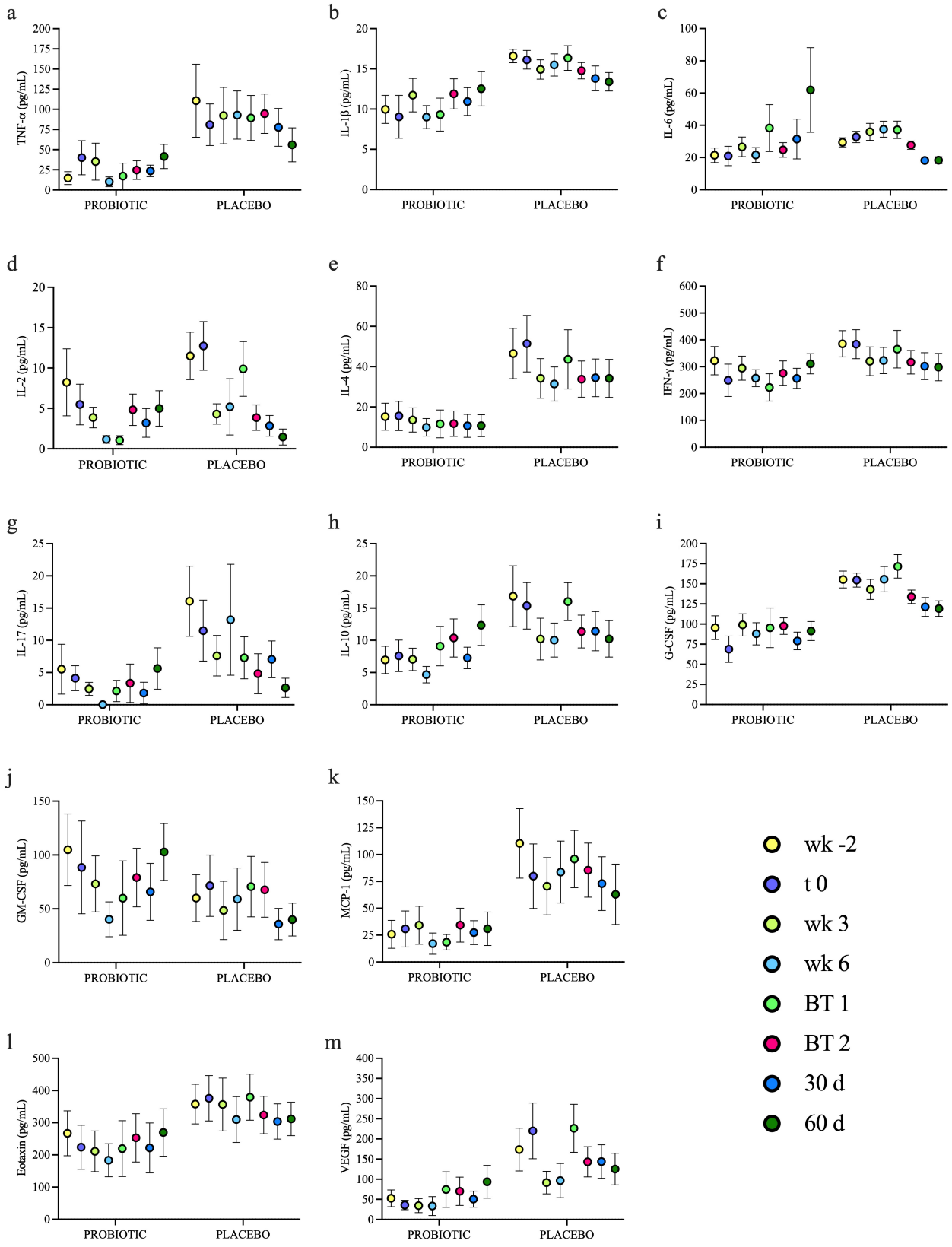

FIGURE 3. Cytokine profile in LACC patients. Serum concentration of (a) TNF-α; (b) IL-1β; (c) IL-6; (d) IL-2; (e) IL-4; (f) IFN-γ; (g) IL-17; (h) IL-10; (i) G-CSF; (j) GM-CSF; (k) MCP-1; (l) eotaxin; and (m) VEGF during treatment in LACC patients from probiotic (n = 15) and placebo (n = 14) groups. Mean ± SEM. Colors representing each time point are indicated. BT: Brachytherapy; TNF: Tumor Necrosis Factor; IL: Interleukin; IFN: Interferon; G-CSF: Granulocyte Colony-Stimulating Factor; GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor; MCP: Monocyte Chemoattractant Protein; VEGF: Vascular Endothelial Growth Factor.

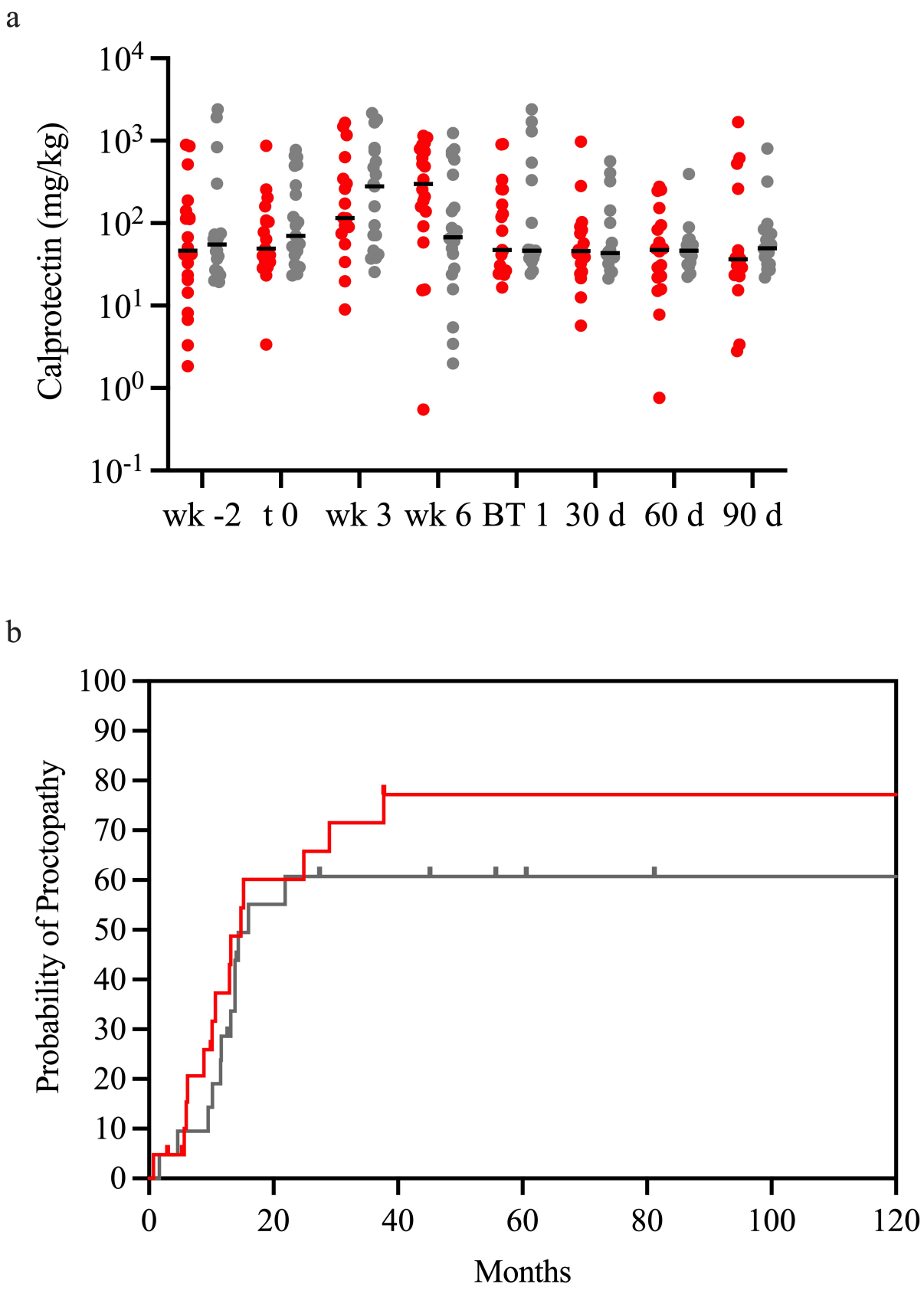

FIGURE 4. Intestinal inflammation in LACC patients. Fecal calprotectin and proctopathy in LACC patients from probiotic (n = 21) and placebo (n = 21) groups. (a) Scatter dot plot of fecal calprotectin comparing the probiotic group (red dots) and placebo group (gray dots). The line represents the median. (b) Probability of proctopathy development after treatment completion. Fraction of patients from the probiotic group (red line) compared to the placebo group (gray line) with a biopsy-confirmed diagnosis of proctopathy. No differences were observed between groups ($p = 0.427$). Log-rank test. BT: Brachytherapy.

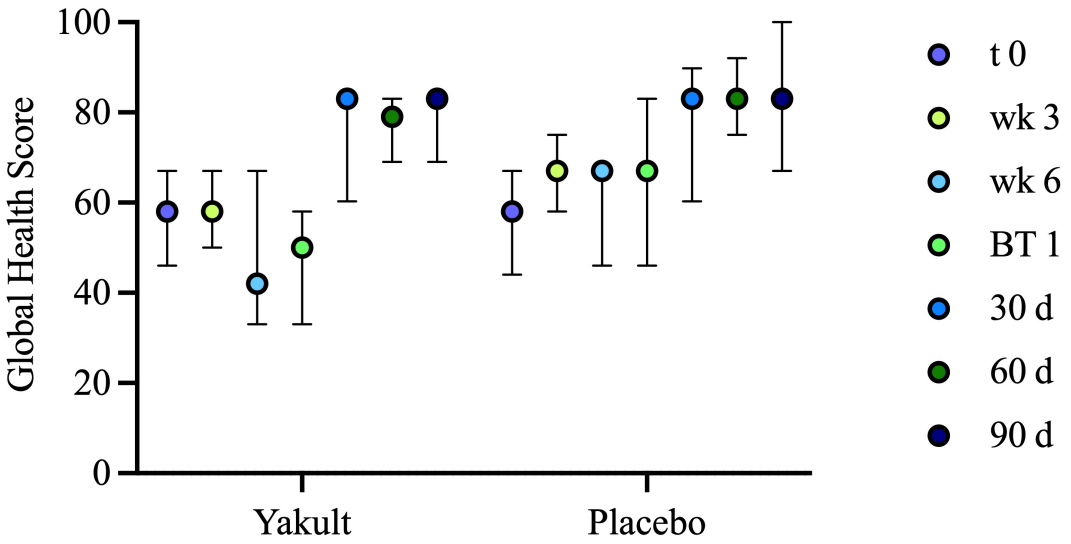

FIGURE 5. Quality of life in LACC. Quality of Life assessment in LACC patients from the probiotic (n = 21) and placebo (n = 21) groups. Median ± IQR of global health score comparing probiotic and placebo groups during treatment (wk 6 and BT 1, $p < 0.05$). Mann Whitney U test. BT: Brachytherapy.

that our intervention with a fermented dairy beverage helped reduce the frequency and severity of nausea and vomiting in the first weeks of CRT. Other studies do not prioritize these symptoms as they focus more on diarrhea [22]. Thus, it is important to note that the fermented beverage helped reduce nausea and vomiting in CC patients. Severe diarrhea is one of the symptoms most related to CRT treatment, and loss of fluids, electrolytes, and nutritional deficiencies increase the risk of severe dehydration [45]. Some studies suggest probiotics adequately prevent CT-related diarrhea, particularly its severity [21, 46]. Clinical recommendations to avoid fermented foods, rich in probiotics and prebiotics, to prevent diarrhea are still habitual practices. However, our results show that patients in the fermented beverage group did not present exacerbation of diarrhea, and it seemed less severe than in the placebo group. A similar randomized clinical trial by Giralt *et al.* [20] analyzed the incidence of radiation-induced diarrhea in gynecologic cancer patients who received an intervention with a probiotic drink containing *Lactobacillus casei*. Compared to the placebo group, the authors reported that the probiotic drink did not reduce the incidence or severity of diarrhea. These findings are consistent with what we observed in our study.

Cancer treatment profoundly impacts the nutritional status of LACC patients [7]. Several factors contribute to the deterioration of the nutritional status. Still, gastrointestinal toxicity symptoms are among the most critical as they lead to reduced food intake (*e.g.*, anorexia, nausea) and nutrient absorption (*e.g.*, vomiting, diarrhea) [46, 47]. As other studies have described [6, 7, 48], we observed continuous weight loss in patients from both groups during treatment, followed by recovery after treatment. No differences were observed among groups despite differences in the symptoms developed. As expected, an increase in inflammatory populations was observed during treatment, as demonstrated by the NLR, though not different among groups.

IL-1β, TNF-α and IL-6 are secreted mainly by innate immune cell populations [49]. These cytokines have a systemic endocrine effect and mediate numerous physiologic alterations during an inflammatory response [50]. For instance, in the hypothalamus, IL-1β and IL-6 induce anorexia and fever. In the liver, TNF-α, IL-1β and IL-6 promote the acute phase response. In skeletal muscle, TNF-α and IL-6 induce muscle catabolism and, consequently, sarcopenia. Part of the systemic metabolic consequences of the effect of these cytokines is related to the obtention of glucose and amino acids, required in abundance for the immune cell effector functions. Unsurprisingly, in cancer, the tumor produces these cytokines [51–53], resulting in a catabolic and inflammatory state. Our study observed that TNF-α was secreted in higher quantities than IL-1β or IL-6. No apparent difference was observed between the groups. A study by Yoshida *et al.* [54] found that the expression of IL-6, TNF-α and IL-1β in patients with renal cell carcinoma before treatment was significantly higher than in healthy controls. In renal cell carcinoma, a different study by Dosquet *et al.* [55] revealed a positive predictive value of 91% for a metastatic spread in patients with IL-6 plasma values above 40 pg/mL. Another study in prostate cancer patients demonstrated similar levels of IL-6 and TNF-α [56]. Comparatively, LACC patients have much higher concentrations of these cytokines. A meta-analysis by Lippitz *et al.* [57] demonstrated a correlation between IL-6 and prognosis in cancer patients. This analysis shows that cutoff values for IL-6 levels vary significantly among studies in different tumor types, ranging from 4 pg/mL in metastatic renal cell carcinoma to 55 pg/mL in metastatic breast cancer. It is important to note that this study did not include LACC patients. A study by Liu *et al.* [58] reported that in LACC patients, the median concentration of IL-6 was 10 pg/mL before treatment compared to 15 pg/mL after treatment. Therefore, the expression levels of these cytokines in our LACC patient population suggest a critical systemic inflammatory state.

We also analyzed the expression of immune cell growth factors and chemokines. G-CSF and GM-CSF are myeloid growth factors that stimulate the production of granulocytes in bone marrow, mainly neutrophils and macrophages [59]. During cancer treatment, an adverse effect of CRT is the suppression of hematopoiesis, so the production of G-CSF and GM-CSF would benefit patients suffering from neutropenia and monocytopenia [59]. In our study, these factors are expressed in both groups of patients; G-CSF is expressed more abundantly in the placebo group at several points in time. Concordantly, an increase in NLR was observed during treatment in all patients. MCP-1 and eotaxin are chemoattractant molecules that recruit monocytes and eosinophils, respectively [60]. High levels of eotaxin have been associated with postoperative complications in colorectal cancer patients [61]. MCP-1 involves monocyte/macrophage migration and infiltration into inflamed tissues in many chronic disorders [60]. We observed a higher expression of eotaxin and MCP-1 in patients from the placebo group before and during treatment. These results suggest that our patient population is undergoing systemic inflammation with a potential infiltration of innate immune cells to inflamed tissues.

To understand the adaptive immune response in patients, we analyzed the levels of several cytokines, including Th1, Th2 and Th17 cytokines [62]. IFN-γ was notably expressed in both groups of patients, IL-4 seemed higher in the placebo group, and levels of IL-2 and IL-17 were comparatively low in both groups of patients. A study by Liu *et al*. [63] correlated alterations in Th17 cells in LACC patients with CRT treatment efficacy. A decrease in circulating Th17 cells after CRT correlated with longer Progression Free Survival and OS. Th1 responses are anti-tumoral immune responses associated with good prognosis and clinical response [64]. However, the high expression of IFN-γ we observed, is not reflected in the tumor; therefore, we cannot presume that this immune effector response is taking place in the tumor microenvironment of these patients. Also, the tumor is not the only tissue being damaged by the treatment; other healthy tissues suffer toxicity, contributing to the inflammatory phenomena described.

Regulatory T cells are the primary producers of IL-10, which has a potent anti-inflammatory response [65]. In this study, we observed that all patients had a low expression of IL-10, and we did not observe differences among groups. This finding suggests that the patients present a high inflammatory status, and no apparent anti-inflammatory mechanisms are being developed to compensate. It is important to remember that initially, the tumor may induce inflammation, and the cancer treatment causes critical levels of acute inflammation in response to damage to the tumor and nearby healthy tissues [62].

Systemic inflammation does not always reflect the localized inflammatory response. Activated inflammatory neutrophils in the gut release fecal calprotectin, reflecting an acute inflammatory response in the intestinal mucosa [66, 67]. For this reason, we analyzed fecal calprotectin levels. In all patients, calprotectin levels are increased during CRT treatment and improve after treatment completion. Considering that no significant differences were described among groups, we did observe a trend towards higher secretion of calprotectin in the placebo group, except for week 6 of CRT and 90 days after treatment, the same time points where we observed a higher frequency and severity of proctitis in patients from the probiotic group.

Interestingly, quantifying fecal calprotectin is a useful marker of intestinal inflammation and toxicity symptoms [68]. de Loera-Rodríguez *et al*. [69] analyzed fecal calprotectin in Mexican LACC patients who received symbiotic supplementation with the probiotics *Lactobacillus acidophilus*, Bifidobacterium *lactis* and prebiotic inulin. The authors reported a significant decrease in calprotectin levels at week seven in patients who received the symbiotic, compared to placebo. The fecal calprotectin levels reported by de Loera-Rodríguez are higher at baseline than what we found in our study patients. Still, their results for week 4 were very similar to our data during CRT treatment on week 3. By week seven, they reported a significant drop in calprotectin levels in the symbiotic group but not in the placebo group. Still, our study shows much lower calprotectin levels by the end of CRT. A possible explanation of the differences observed may be treatment modalities; unfortunately, the authors did not describe the treatment characteristics of their groups of patients. This study also analyzed gastrointestinal toxicity and found less frequency and severity of vomiting in the symbiotic group compared to the placebo. This result is consistent with our findings of significantly lower frequency and severity of vomiting in patients in the probiotic group.

Finally, VEGF is a growth factor that promotes angiogenesis and tumor invasion, progression, and metastasis [70]. Our patients showed a high expression of this molecule. In LACC, VEGF expression has been reported, and treatment with Bevacizumab, a monoclonal antibody directed against VEGF, is indicated in persistent and recurrent disease [71]. In our study, we confirmed the presence of VEGF in the LACC patient population and, importantly, in seemingly lower levels in the probiotic group.

The acute inflammatory condition in LACC patients led us to question if it would evolve into chronic toxicity and its impact on health-related quality of life. Thus, we analyzed the development of proctopathy. Patients who consumed the fermented dairy beverage tended to develop more proctopathy than patients in the placebo group, even though no statistically significant difference was observed. This result is related to the trend of a small percentage of patients in the intervention group developing grade 3 proctitis after treatment. This result suggests a possible adverse response to probiotics in the context of a fermented dairy beverage. It is important to note that this was a short-term intervention; patients stopped consuming the fermented beverage 90 days post-treatment. We believe that diet is a crucial factor when analyzing trials with probiotic interventions, and in this case, the patient's diet was not evaluated after the study's conclusion. In particular, fiber is a dietary component that may determine the establishment of probiotic populations in the host. Fiber is a prebiotic and substrate for butyrate production, with a demonstrated anti-inflammatory effect [72, 73]. Further studies need to address fiber consumption and other dietary components to discern the impact of probiotic supplementation in its interaction with prebiotics and the production of fermentation-derived metabolites.

Another possible mechanism is related to the pathophysiologic effect of RT on the intestine [74]. RT induces cell death in the enterocytes and colonocytes. This disruption in the intestinal barrier may render it unable to tolerate the bacterial load from the fermented beverage. Thus, it is possible that instead of contributing to a tolerogenic anti-inflammatory environment, the presence of bacteria and derived metabolites reach the lamina propria and induce an inflammatory response. This theory is further supported by the high expression of fecal calprotectin observed at certain times during and after treatment. Another aspect to consider regarding the toxicity reported in this study is that patients received RT treatment with cobalt-60, which may induce a severe inflammatory response and toxicity, as stated above.

To our knowledge, the relationship between probiotics and quality of life in LACC patients has yet to be evaluated. Some studies have assessed the quality of life in LACC patients using the EORTC QLQ-C30 and CX24 instruments and their association with treatment response and survival. These studies confirmed that CC harms the quality of life, causing a decrease in the physical and mental domains in the development of depressive symptoms, vitality, social functioning and overall health reported in CC patients [75–77]. The health-related quality of life of women who suffer from LACC improves after CRT treatment, mainly in the global health score. Improvement in the areas related to symptoms such as fatigue, pain and emotional and economic problems has been reported [78]. Evidence regarding the impact of probiotics on quality-of-life scores is still insufficient; however, none of the studies reported severe side effects, so including probiotics seems safe [17].

5. Conclusions

In conclusion, our study demonstrated the beneficial effect of *Lactobacillus casei Shirota*-based fermented dairy beverages on reducing the frequency and severity of vomiting. Still, it did not provide benefits regarding other treatment-related toxicities, including diarrhea. In addition, cytokine expression changes were observed, suggesting a role in the modulation of the immune response—however, a clear trend needs to be demonstrated. Therefore, we propose that using *Lactobacillus casei Shirota*-based fermented dairy beverage during CRT is safe and may benefit LACC patients. Of course, more research on using fermented products as probiotic sources and their benefit for LACC patients needs to be generated to establish evidence-based recommendations. Dietary patterns, microbiota, and derived metabolites contribute to maintaining health or developing disease and must be addressed in trials that study probiotic interventions.

6. Study limitations

Our study's limitations included the small sample size and the recruitment period from 2006 to 2008, which may be regarded as old. Nonetheless, we deemed it important to establish the safety of probiotic supplementation and the development of chronic symptoms like proctopathy in this group of patients immunocompromised by cancer treatment. Another limitation was that the patients in the probiotic group were significantly older and uneducated compared to the placebo group. Age may impact the inflammatory response, and a fragile state may confer a risk for increased toxicity.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

LdCCP—Conceptualization; funding acquisition; project administration; resources. JLM, MDG, ARE—Data curation. DCE, JLM, MDG, ARE—Formal analysis. CAM, CCH—Investigation. CCH, AEN, MGST—Methodology. LdCCP, DCE—Supervision. DCE, JLM—Validation. DCE—Visualisation; writing-review & editing. DCE, JLM, MDG—Writing-original draft. All authors reviewed the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Subjects have given their written informed consent, and the study protocol was approved by the Research and Ethics Committees of the Instituto Nacional de Cancerología (008/043/ICI) (CB470). All procedures were carried out per the 1964 Helsinki Declaration and its later amendments. Participants voluntarily agreed to participate in the study and signed an informed consent. Trial Registration Number NCT05736315 was retrospectively registered on 16 February 2023.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, *et al*. Global cancer observatory: cancer today. International agency for

research on cancer. 2022. Available at: https://gco.iarc.fr/en (Accessed: 06 December 2024).

[2] Bhatla N, Aoki D, Sharma DN, Sankaranarayanan R. Cancer of the cervix uteri. International Journal of Gynecology Obstetrics. 2018; 143: 22–36.

[3] Lehman M, Thomas G. Is concurrent chemotherapy and radiotherapy the new standard of care for locally advanced cervical cancer? International Journal of Gynecological Cancer. 2001; 11: 87–99.

[4] Roswit B, Malsky SJ, Reid CB. Severe radiation injuries of the stomach, small intestine, colon and rectum. The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine. 1972; 114: 460–475.

[5] Wu N, Bu M, Jiang H, Mu X, Zhao H. Dose-effect relationship in external beam radiotherapy combined with brachytherapy for cervical cancer: a systematic review. Journal of Contemporary Brachytherapy. 2024; 16: 232–240.

[6] Morris KA, Haboubi NY. Pelvic radiation therapy: between delight and disaster. World Journal of Gastrointestinal Surgery. 2015; 7: 279–288.

[7] Sánchez M, Castro-Eguiluz D, Luvián-Morales J, Jiménez-Lima R, Aguilar-Ponce JL, Isla-Ortiz D, *et al*. Deterioration of nutritional status of patients with locally advanced cervical cancer during treatment with concomitant chemoradiotherapy. Journal of Human Nutrition and Dietetics. 2019; 32: 480–491.

[8] Tian M, Xu H, Wang H, Wang H, Dai Z, Ding C, *et al*. Pretreatment computed tomography–defined sarcopenia, treatment-associated muscle loss, and survival in patients with cervical cancer: a systematic review and meta-analysis. Nutrition Reviews. 2025; 83: 797–808.

[9] Medina-Contreras O, Luvián-Morales J, Valdez-Palomares F, Flores-Cisneros L, Sánchez-López M, Soto-Lugo JH, *et al*. Immunonutrition in cervical cancer: immune response modulation by diet. Revista de Investigacion Clinica. 2020; 72: 219–230.

[10] Ren H, Wu Q, Sun Z, Fang M, Liu J, Luo J. Research progress and treatment of radiation enteritis and gut microbiota. Radiation Oncology Journal. 2023; 41: 61–68.

[11] Mitra A, Grossman Biegert GW, Delgado AY, Karpinets TV, Solley TN, Mezzari MP, *et al*. Microbial diversity and composition is associated with patient-reported toxicity during chemoradiation therapy for cervical cancer. International Journal of Radiation Oncology, Biology, Physics. 2020; 107: 163–171.

[12] Hibberd AA, Lyra A, Ouwehand AC, Rolny P, Lindegren H, Cedgård L, *et al*. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. BMJ Open Gastroenterology. 2017; 4: e000145.

[13] Reid G. Probiotics: Definition, scope and mechanisms of action. Best Practice & Research Clinical Gastroenterology. 2016; 30: 17–25.

[14] George Kerry R, Patra JK, Gouda S, Park Y, Shin HS, Das G. Benefaction of probiotics for human health: a review. Journal of Food and Drug Analysis. 2018; 26: 927–939.

[15] Faria AMC, Reis BS, Mucida D. Tissue adaptation: Implications for gut immunity and tolerance. The Journal of Experimental Medicine. 2017; 214: 1211–1226.

[16] La Fata G, Weber P, Mohajeri MH. Probiotics and the gut immune system: indirect regulation. Probiotics and Antimicrobial Proteins. 2018; 10: 11–21.

[17] Gutiérrez Salmeán G, Delgadillo González M, Rueda Escalona AA, Leyva Islas JA, Castro-Eguiluz D. Effects of prebiotics, probiotics, and synbiotics on the prevention and treatment of cervical cancer: Mexican consensus and recommendations. Frontiers in Oncology. 2024; 14: 1383258.

[18] Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. Advances in Nutrition. 2019; 10: S49–S66.

[19] Serban DE. Gastrointestinal cancers: Influence of gut microbiota, probiotics and prebiotics. Cancer Letters. 2014; 345: 258–270.

[20] Giralt J, Regadera JP, Verges R, Romero J, de la Fuente I, Biete A, *et al*. Effects of probiotic Lactobacillus casei DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. International Journal of Radiation Oncology, Biology, Physics. 2008; 71: 1213–1219.

[21] Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, Lorvidhaya V. Randomized controlled trial of live lactobacillus acidophilus plus bifidobacterium bifidum in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. Radiation Oncology. 2010; 5: 31.

[22] Delia P, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, *et al*. Use of probiotics for prevention of radiation-induced diarrhea. World Journal of Gastroenterology. 2007; 13: 912–915.

[23] Şanlier N, Gökcen BB, Sezgin AC. Health benefits of fermented foods. Critical Reviews in Food Science and Nutrition. 2019; 59: 506–527.

[24] Aslam H, Green J, Jacka FN, Collier F, Berk M, Pasco J, *et al*. Fermented foods, the gut and mental health: a mechanistic overview with implications for depression and anxiety. Nutritional Neuroscience. 2020; 23: 659–671.

[25] Wastyk HC, Fragiadakis GK, Perelman D, Dahan D, Merrill BD, Yu FB, *et al*. Gut-microbiota-targeted diets modulate human immune status. Cell. 2021; 184: 4137–4153.e14.

[26] Shiby VK, Mishra HN. Fermented milks and milk products as functional foods—a review. Critical Reviews in Food Science and Nutrition. 2013; 53: 482–496.

[27] Ashraf R, Shah NP. Immune system stimulation by probiotic microorganisms. Critical Reviews in Food Science and Nutrition. 2014; 54: 938–956.

[28] Dong H, Rowland I, Tuohy KM, Thomas LV, Yaqoob P. Selective effects of *Lactobacillus casei* Shirota on T cell activation, natural killer cell activity and cytokine production. Clinical and Experimental Immunology. 2010; 161: 378–388.

[29] Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M, Okumura K. Interleukin-12 is involved in the enhancement of human natural killer cell activity by *Lactobacillus casei* Shirota. Clinical and Experimental Immunology. 2006; 146: 109–115.

[30] Harbige LS, Pinto E, Allgrove J, Thomas LV. Immune response of healthy adults to the ingested probiotic *Lactobacillus casei* Shirota. Scandinavian Journal of Immunology. 2016; 84: 353–364.

[31] Vaisberg M, Paixão V, Almeida EB, Santos JMB, Foster R, Rossi M, *et al*. Daily intake of fermented milk containing *Lactobacillus casei* Shirota (Lcs) modulates systemic and upper airways immune/inflammatory responses in marathon runners. Nutrients. 2019; 11: 1678.

[32] Lv L, Ren S, Jiang H, Yan R, Chen W, Yan R, *et al*. The oral administration of *Lacticaseibacillus casei* Shirota alleviates acetaminophen-induced liver injury through accelerated acetaminophen metabolism via the liver-gut axis in mice. mSphere. 2024; 9: e0067223.

[33] Foey A, Habil N, Strachan A, Beal J. *Lacticaseibacillus casei* Strain Shirota modulates macrophage-intestinal epithelial cell co-culture barrier integrity, bacterial sensing and inflammatory cytokines. Microorganisms. 2022; 10: 2087.

[34] García-Peris P, Velasco C, Lozano MA, Moreno Y, Paron L, de la Cuerda C, *et al*. Effect of a mixture of inulin and fructo-oligosaccharide on Lactobacillus and Bifidobacterium intestinal microbiota of patients receiving radiotherapy: a randomised, double-blind, placebo-controlled trial. Nutrición Hospitalaria. 2012; 27: 1908–1915.

[35] Wong S, Hirani SP, Forbes A, Kumar N, Hariharan R, O'Driscoll J, *et al*. *Lactobacillus casei* Shirota probiotic drinks reduce antibiotic associated diarrhoea in patients with spinal cord injuries who regularly consume proton pump inhibitors: a subgroup analysis of the ECLISP multicentre RCT. Spinal Cord. 2024; 62: 255–263.

[36] Cook CM, Makino H, Kato K, Blonquist T, Derrig L, Shibata H. The probiotic Lacticaseibacillus paracasei strain Shirota (LcS) in a fermented milk beverage survives the gastrointestinal tract of generally healthy U.S. Adults. International Journal of Food Sciences and Nutrition. 2023; 74: 645–653.

[37] Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, *et al*. ESPEN guidelines on nutrition in cancer patients. Clinical Nutrition. 2017; 36: 11–48.

[38] Chiswick EL, Duffy E, Japp B, Remick D. Detection and quantification of cytokines and other biomarkers. Methods in Molecular Biology. 2012; 844: 15–30.

[39] Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, *et al*. The European Organization for research and treatment of cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. Journal of the National Cancer Institute. 1993; 85: 365–376.

[40] Indaram AV, Visvalingam V, Locke M, Bank S. Mucosal cytokine production in radiation-induced proctosigmoiditis compared with inflammatory bowel disease. The American Journal of Gastroenterology. 2000;

95: 1221–1225.

[41] Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: a review. Radiation Research. 2012; 178: 505–523.

[42] Gerassy-Vainberg S, Blatt A, Danin-Poleg Y, Gershovich K, Sabo E, Nevelsky A, *et al*. Radiation induces proinflammatory dysbiosis: transmission of inflammatory susceptibility by host cytokine induction. Gut. 2018; 67: 97–107.

[43] Sammour T, Kahokehr AA. Pelvic radiation disease. Clinics in Colon and Rectal Surgery. 2022; 35: 204–211.

[44] Bhatia M, Suliman H, Ahmed R, Kostadinov D, Singhal T. Radiation proctitis: a review of pathophysiology and treatment strategies. Cureus. 2024; 16: e70581.

[45] Arbuckle RB, Huber SL, Zacker C. The consequences of diarrhea occurring during chemotherapy for colorectal cancer: a retrospective study. The Oncologist. 2000; 5: 250–259.

[46] Garczyk A, Kaliciak I, Drogowski K, Horwat P, Kopeć S, Staręga Z, *et al*. Influence of probiotics in prevention and treatment of patients who undergo chemotherapy or/and radiotherapy and suffer from mucositis, diarrhoea, constipation, nausea and vomiting. Journal of Clinical Medicine. 2022; 11: 3412.

[47] Tamayo-Torres E, Garrido A, de Cabo R, Carretero J, Gómez-Cabrera MC. Molecular mechanisms of cancer cachexia. Role of exercise training. Molecular Aspects of Medicine. 2024; 99: 101293.

[48] Saibishkumar EP, Patel FD, Sharma SC. Evaluation of late toxicities of patients with carcinoma of the cervix treated with radical radiotherapy: an audit from India. Clinical oncology. 2006; 18: 30–37.

[49] Tylutka A, Walas Ł, Zembron-Lacny A. Level of IL-6, TNF, and IL-1β and age-related diseases: a systematic review and meta-analysis. Frontiers in Immunology. 2024; 15: 1330386.

[50] Hirano T. IL-6 in inflammation, autoimmunity and cancer. International Immunology. 2021; 33: 127–148.

[51] Wang YF, An ZY, Lin DH, Jin WL. Targeting cancer cachexia: Molecular mechanisms and clinical study. MedComm. 2022; 3: e164.

[52] Yeom E, Yu K. Understanding the molecular basis of anorexia and tissue wasting in cancer cachexia. Experimental & Molecular Medicine. 2022; 54: 426–432.

[53] Paval DR, Patton R, McDonald J, Skipworth RJE, Gallagher IJ, Laird BJ. A systematic review examining the relationship between cytokines and cachexia in incurable cancer. Journal of Cachexia, Sarcopenia and Muscle. 2022; 13: 824–838.

[54] Yoshida N, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, *et al*. Interleukin-6, tumour necrosis factor α and interleukin-1β in patients with renal cell carcinoma. British Journal of Cancer. 2002; 86: 1396–1400.

[55] Dosquet C, Schaetz A, Faucher C, Lepage E, Wautier JL, Richard F, *et al*. Tumour necrosis factor-α, interleukin-1β and interleukin-6 in patients with renal cell carcinoma. European Journal of Cancer. 1994; 30A: 162–167.

[56] Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. British Journal of Cancer. 2004; 90: 2312–2316.

[57] Lippitz BE, Harris RA. Cytokine patterns in cancer patients: a review of the correlation between interleukin 6 and prognosis. Oncoimmunology. 2016; 5: e1093722.

[58] Liu X, Meng L, Chen L, Liang Y, Wang B, Shao Q, *et al*. IL-6 expression promoted by Poly(I:C) in cervical cancer cells regulates cytokine expression and recruitment of macrophages. Journal of Cellular and Molecular Medicine. 2020; 24: 2284–2293.

[59] Mehta HM, Malandra M, Corey SJ. G-CSF and GM-CSF in neutropenia. The Journal of Immunology. 2015; 195: 1341–1349.

[60] Singh S, Anshita D, Ravichandiran V. MCP-1: function, regulation, and involvement in disease. International Immunopharmacology. 2021; 101: 107598.

[61] Zajkowska M, Mroczko B. Eotaxins and their receptor in colorectal cancer—a literature review. Cancers. 2020; 12: 1383.

[62] Wang H, Wang T, Yan S, Tang J, Zhang Y, Wang L, *et al*. Crosstalk of pyroptosis and cytokine in the tumor microenvironment: from mechanisms to clinical implication. Molecular Cancer. 2024; 23: 268.

[63] Liu Y, Guo QF, Chen JL, Li XR, Hou F, Liu XY, *et al*. Correlations between alterations of T-helper 17 cells and treatment efficacy after concurrent radiochemotherapy in locally advanced cervical cancer (stage IIB–IIIB): a 3-year prospective study. Chinese Medical Journal. 2021; 134: 954–962.

[64] Belardelli F, Ferrantini M. Cytokines as a link between innate and adaptive antitumor immunity. Trends in Immunology. 2022; 23: 201–208.

[65] Singer M, Elsayed AM, Husseiny MI. Regulatory T-cells: the face-off of the immune balance. Frontiers in Bioscience. 2024; 29: 377.

[66] Ross FA, Park JH, Mansouri D, Combet E, Horgan PG, McMillan DC, *et al*. The role of faecal calprotectin in diagnosis and staging of colorectal neoplasia: a systematic review and meta-analysis. BMC Gastroenterology. 2022; 22: 176.

[67] Kapel N, Ouni H, Benahmed NA, Barbot-Trystram L. Fecal calprotectin for the diagnosis and management of inflammatory bowel diseases. Clinical and Translational Gastroenterology. 2023; 14: e00617.

[68] Ling Lundström M, Peterson C, Hedin CRH, Bergemalm D, Lampinen M, Magnusson MK, *et al*. Faecal biomarkers for diagnosis and prediction of disease course in treatment-naïve patients with IBD. Alimentary Pharmacology & Therapeutics. 2024; 60: 765–777.

[69] De Loera Rodríguez LH, Ortiz GG, Rivero Moragrega P, Velázquez Brizuela IE, Santoscoy Gutiérrez JF, Rincón Sánchez AR, *et al*. Effect of symbiotic supplementation on fecal calprotectin levels and lactic acid bacteria, Bifidobacteria, Escherichia coli and Salmonella DNA in patients with cervical cancer. Nutrición Hospitalaria. 2018; 35: 1394–1400.

[70] Patel SA, Nilsson MB, Le X, Cascone T, Jain RK, Heymach JV. Molecular mechanisms and future implications of VEGF/VEGFR in cancer therapy. Clinical Cancer Research. 2023; 29: 30–39.

[71] National Comprehensive Cancer Network. Cervical cancer-guidelines for patients details. 2024. Available at: https://www.nccn.org/patientresources/patient-resources/guidelines-for-patients/guidelines-for-patients-details?patientGuidelineId=62 (Accessed: 06 December 2024).

[72] Then CK, Paillas S, Moomin A, Misheva MD, Moir RA, Hay SM, *et al*. Dietary fibre supplementation enhances radiotherapy tumour control and alleviates intestinal radiation toxicity. Microbiome. 2024; 12: 89.

[73] Parada Venegas D, De la Fuente MK, Landskron G, González MJ, Quera R, Dijkstra G, *et al*. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Frontiers in Immunology. 2019; 10: 277.

[74] Yi Y, Lu W, Shen L, Wu Y, Zhang Z. The gut microbiota as a booster for radiotherapy: novel insights into radio-protection and radiation injury. Experimental Hematology & Oncology. 2023; 12: 48.

[75] Klapheke AK, Keegan THM, Ruskin R, Cress RD. Changes in health-related quality of life in older women after diagnosis with gynecologic cancer. Gynecologic Oncology. 2020; 156: 475–481.

[76] Kumari A, Panigrahi A, Roy A, Panda J. Impaired quality of life and its determinants among postmenopausal women of slum communities in Bhubaneswar, India. Journal of Mid-Life Health. 2020; 11: 149–155.

[77] Luvián-Morales J, Flores-Cisneros L, Jiménez-Lima R, Alarcón-Barrios S, Salazar-Mendoza J, Castro-Eguiluz D, *et al*. Validation of the QLQ-CX24 questionnaire for the assessment of quality of life in Mexican women with cervical cancer. International Journal of Gynecologic Cancer. 2021; 31: 1228–1235.

[78] Laky B, Janda M, Kondalsamy-Chennakesavan S, Cleghorn G, Obermair A. Pretreatment malnutrition and quality of life—association with prolonged length of hospital stay among patients with gynecological cancer: a cohort study. BMC Cancer. 2010; 10: 232.

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