Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-018-9408-9

Effect of Probiotics for the Prevention of Acute Radiation-Induced Diarrhoea Among Cervical Cancer Patients: a Randomized Double-Blind Placebo-Controlled Study

Ye Htut Linn[1] · K. Khine Thu[1] · Nang Hla Hla Win[1]

Abstract
Radiotherapy is the mainstay treatment of cervical cancer and the most common acute side effect is radiation-induced diarrhoea (RID), which can affect up to 80% of the patients. The most frequently used probiotics for the RID in previous studies with somewhat positive results are *Lactobacillus* and *Bifidobacterium* strains. This study was aimed to investigate the effect of a probiotic containing live *Lactobacillus acidophilus* LA-5 plus *Bifidobacterium animalis* subsp. *lactis* BB-12 for the prevention of acute (RID) among cervical cancer patients. Patients receiving external beam pelvic radiotherapy with or without concurrent chemotherapy were randomized into probiotic or placebo groups and were double-blinded. The probiotic group received the capsules containing 1.75 billion lyophilized live bacteria to be taken one capsule three times daily beginning from the first day until the end of radiotherapy, and the placebo group received identically appearing capsules containing starch with the same schedule. Every patient received the standard dietary recommendations. The patients were assessed daily during radiotherapy and follow-up weekly for 3 weeks after radiotherapy. Total 54 patients were analyzed. The incidence of diarrhoea was reduced in the probiotic group than the placebo group (53.8 and 82.1%, $p < 0.05$). The mild-to-moderate and severe diarrhoea were significantly reduced in the probiotic group ($p < 0.05$). The use of loperamide as an anti-diarrhoeal medication was significantly reduced in the probiotic group than the placebo group ($p < 0.01$). The difference in grade 2 abdominal pain and episodes of abdominal pain in days were also significant ($p < 0.001$). Therefore, supplementation of probiotic is an easy and effective way to reduce the incidence and severity of RID in cervical cancer patients.

Keywords Probiotics · *Lactobacillus* · *Bifidobacterium* · Radiation-induced diarrhoea · Cervical cancer

Introduction

Globally, cancers are one of the leading causes of morbidity and mortality and there were approximately 14 million new cases of cancers and 8.2 million cancers related death in 2012. Among these cancers, cervical cancer is the fourth most commonest and fourth highest mortality rate among cancer in women worldwide [1]. According to the Human Papillomavirus and Related Cancers, Fact Sheet 2016, Myanmar has 20.19 million women ages 15 years and older who are at risk of developing cervical cancer. It is estimated that every year, 5286 women are diagnosed with cervical cancer and 2998 die from the disease. Cervical cancer was the second most common cancer among women in Myanmar and the first most common cancer among women aged between 15 to 44 years [2]. In Department of Radiotherapy of Yangon General Hospital (YGH), cervical cancer was the second leading cause of morbidity among women, ranked third in both sex and there was 1027 registered new cases in 2016 [3].

As most of the cervical cancers are radiosensitive, radiation therapy is the mainstay treatment option in standard treatment of cervical cancer. Starting from the FIGO (Féderation Internationale de Gynécologie et d'Obstétrique) stage I B,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12602-018-9408-9) contains supplementary material, which is available to authorized users.

✉ Ye Htut Linn
dryehtutlinn@gmail.com

K. Khine Thu
drkkt2011@gmail.com

Nang Hla Hla Win
nanhlahlawin@gmail.com

[1] Department of Pharmacology, University of Medicine 1, Yangon. No. 245, Myoma Kyaung Street, Lanmadaw Township, Yangon, Myanmar

Published online: 17 March 2018

external beam pelvis radiotherapy is used alone or in combination with brachytherapy or chemotherapy for both curative and palliative purposes. Radiation-induced bowel damage was first described in 1897 by David Walsh [4]. Gastrointestinal side effects are common in radiotherapy patients, and it is estimated that over 70% will develop acute symptoms whereas less than 5–30% of the patients will experience chronic effects which can adversely affect the quality of life [5, 6]. Diarrhoea is the most common acute side effect of pelvic radiation, and it can occur in up to 80% of the patients [7]. In YGH, acute reaction of the intestine occurred in 62% of the cervical cancer patients who received pelvis radiotherapy [8]. With diarrhoea, malabsorption, mucoid rectal discharge, rectal pain and rectal bleeding can also occur. Chronic effects include bowel obstruction, fistulation, perforation and intractable bleeding. These conditions may be due to an inflammatory response to irradiation, a modification of the intestinal microflora and the flattening of intestinal microvilli which decreases enzymatic activity, reduces absorptive surface area and decreases total gut transit time [6, 9, 10]. The severity of acute bowel toxicity may predetermine the degree to chronic bowel changes [11]. Therefore, it is logical to prevent the incidence or reduce the degree of intestinal toxicity which may have long-term benefits.

Currently, no prophylactic agents have been approved for the prevention of radiation-induced diarrhoea (RID). Attempts to treat and prevent this conditions with 5 amino salicylic acid (5-ASA) and related compounds, amifostine, cholestyramine, formalin, glutamine, hyperbaric oxygen, antibiotics, prostaglandins, octreotide, sodium butyrate, sucralfate, physical activity, heater probes and circadian rhythm have so far provided inconclusive clinical results with failure of treatment occurring in a substantial proportion of patients [12]. According to these contradictory findings, new approaches that target other steps in the pathophysiology of radiation-induced diarrhoea are urgently needed.

According to the World Gastroenterology Organization (WGO) practice guideline, probiotics are defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" [13]. A meta-analysis concluded that probiotic supplementation shows a probable beneficial effect in the prevention and possible benefit in the treatment of RID [14]. It is therefore logical to explore the use of supplementary probiotics in prophylaxis against RID. The possible mechanisms of probiotics may be due to correction of dysbiosis, down-modulation of the severity of intestinal inflammation, down-modulation of apoptosis which is regarded as the main factor responsible for the radiation-induced injury of the intestinal epithelium and up-regulation of the innate immune response in the gut which give protection against intestinal colonization by invasive pathogens. They also stimulate lactase production, which helps lactose digestion because lactase may be reduced or loss due to the damage to the intestinal villi [15, 16].

The most commonly used probiotics for the RID in the previous studies are *Lactobacillus* and *Bifidobacterium* strains and got the somewhat positive results in some studies [17–19]. There were some clinical trials which used probiotics to prevent the RID in pelvic radiotherapy patients. However, their results are somewhat contradictory and difficult to interpret because of differences in included patients, type of cancers, study designs, probiotic stains and formulation and the quality of studies [14]. Therefore, this study was aimed to evaluate the effect of a probiotic containing *Lactobacillus acidophilus* LA-5 plus *Bifidobacterium animalis* subsp. *lactis* BB-12 to reduce the incidence or the severity of RID in cervical cancer patients.

Materials and Methods

This study was planned to investigate the effect of a probiotic containing live *L. acidophilus* LA-5 plus *B. animalis* subsp. *lactis* BB-12 for the prevention of acute RID among cervical cancer patients. The study design was a randomized, double-blind, placebo-controlled study. The flow chart of study procedure is presented in Fig. 1. This study was approved and passed by the Postgraduate Board of Studies (Pharmacology), University of Medicine 1, Yangon (which is an institutional review board (IRB)), and this trial was registered at the Thai Clinical Trial registry with the trial registration number TCTR20170314001.

The cervical cancer patients with FIGO staging I B and above who will undergo external beam pelvic radiotherapy with the standard dose of 50 Gy with or without concurrent chemotherapy were recruited from May, 2016 to November, 2016 at the Department of Radiotherapy, YGH. The patients were above 18 years of age with an Eastern Cooperative Oncology Group (ECOG) performance status of zero to two (see supplementary file 1). Exclusion criteria were as follows: patients with the previous history of pelvic radiotherapy; patients with the history of intestinal resection; patients with the medical history of irritable bowel syndrome, malabsorption syndrome, inflammatory bowel disease and ileostomy; patients with the history of daily use of anti-diarrhoeal medication before radiotherapy; patients with diarrhoea at the beginning of study, patients with immunosuppression (HIV positive, immunosuppressive drugs) and patients with structural heart diseases and those with prosthetic heart valves. After selecting with the inclusion and exclusion criteria, informed consent (see supplementary files 2 and 3) was taken prior to the study. Consented patients were allocated into the probiotic or control groups by randomization and sealed opaque envelope system. Randomization was done by a random number generator that balanced allocation to groups A and B: in successive blocks each containing 10 patients each (see

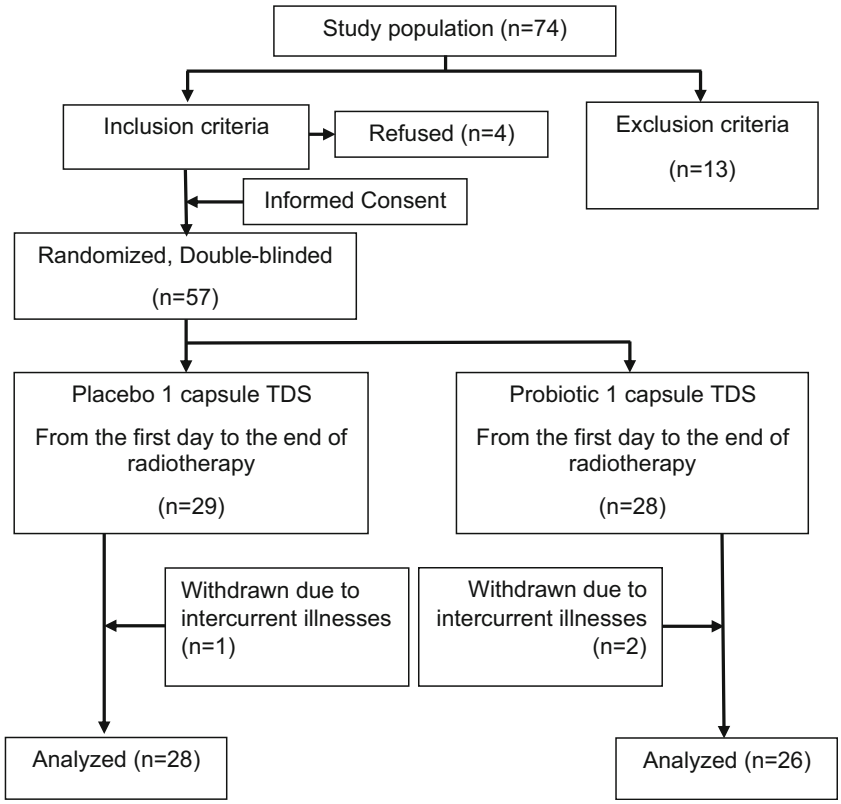

Fig. 1 Flow chart of study procedure (TDS three times a day)

supplementary file 4). Then, the patient number and allocated treatment (group A or B) were put in sealed opaque envelopes. Once a patient has consented to participate in this study, an envelope was opened and the patient was then offered the allocated treatment.

They were given 2D radiotherapy (AP-PA) of five fractions per week for 5 weeks with linear accelerators I and II of YGH (model—CLINAC iX (SN: 5637 and 5638), Varian Medical Systems, USA). The upper margin of the field was defined at the LV_5-SV_1 junction, the lower margin was at 2 cm below the lower tumour extent usually below the obturator foramen and the lateral margin was at 1 cm lateral to the bony pelvis.

The probiotic group was given one capsule (Biogurt®—each capsule contains functional yogurt 300 mg containing 1.75 billion lyophilized live *L. acidophilus* LA-5 plus *B. animalis* subsp. *lactis* BB-12, produced by Fame Pharmaceuticals, Myanmar, reg no.—TMR 011383 (0615), mfd—April, 2016, exp—April, 2018) three times daily, beginning from the first day of radiotherapy as a prophylactic treatment before RID appeared, continuing every day until the end of radiotherapy. The control group received placebo capsules (containing starch of equal weight as the study drug) which had identical colour and size as the study drug. The treatment schedule was also the same as that of the probiotic group. Prepackaged probiotic medication and placebo were blinded and coded (group A or B) by the company, and the coded envelop was kept by the professor/head of department. Neither the patients nor the researcher knew whether the patient was on the study drug or placebo. The patient was required to return their drug bottles weekly, and the number of capsules returned was checked and documented the compliance. Patients who took less than 80% of the drugs were considered as noncompliance. The patients were allowed to take rescue/anti-diarrhoeal medication—loperamide (Dicotil®, each tablet contains loperamide HCl 2 mg, from Picco Pharma, Thailand, MYR reg no.—2010AA8922, L/C No. T—16003, mfd—5.4.2016, exp—5.4.2020) if the diarrhoea occurred more than three times with severe cramping. Loperamide was given as an initial dose of 4 mg by mouth every 4 h, followed by 2 mg by mouth after each unformed stool. Daily total dose should not exceed 16 mg. The patients had to note the time and amount of this medication. Every patient was given the pamphlets that described standard dietary recommendations adapted from the National Cancer Institute (NCI) (see supplementary file 5).

Patients were assessed with the pro forma (see supplementary file 6) daily by face-to-face interview and weekly for 3 weeks after the completion of radiotherapy. The severity of the RID was assessed by the Common Terminology Criteria for Adverse Events (CTCAE version 4.0) (see supplementary file 7), and the severity of abdominal pain was assessed by the CTCAE (version 4.0) (see supplementary file 8). The current study used the CTCAE instead of World Health Organization (WHO) toxicity criteria,

because the CTCAE is more specific for cancer treatments and is more commonly used in oncology studies. The CTCAE grading for diarrhoea and abdominal pain is more accurate than the WHO grading.

Statistical Analysis

Continuous variables were described as mean ± standard deviation and were compared using the independent samples *t* test. Categorical variables were described as percentage and were compared using the chi-squared tests. The *p* values reported are two tailed, and an alpha level of 0.05 was used to assess statistical significance. Data analysis was done on SPSS version 16 (SPSS Inc., 444N. Michigan, Chicago, Illinois, USA). The comparison between cumulative incidences of the time-to-event variables was performed using the Kaplan-Meier curves and log rank (Mantel-Cox) test. Absolute risk reduction (ARR) is the difference between the event rate in the intervention group and that in the control group. Numbers needed to treat (NNT) is the number of patients who need to be treated for one to get the benefit (NNT = 100/ARR).

Results

In this study, a total of 57 patients were recruited from the study period of May, 2016 to November, 2016. They were randomly assigned to the probiotic group ($n = 28$) and the placebo group ($n = 29$). Among them, three patients withdrew from the treatment without completion because of the intercurrent illnesses. As they were excluded from the analysis, a total of 54 patients were included in the analysis. General characteristics of the patients are shown in Tables 1 and 2. In the case of the baseline patients' characteristics, there were no statistically significant differences between the probiotic group and the placebo group.

The incidence of RID is shown in Fig. 2. RID occurred in 14 patients in the probiotic group (53.8%) compared with 23 patients in the placebo group (82.1%). There was a statistically significant reduction of the incidence of RID in the probiotic group ($p = 0.025$). ARR is 28.3%, and numbers needed to treat (NNT) were 3.5.

Mild-to-moderate diarrhoea (grade 1 or 2) occurred in 14 patients (53.8%) of the probiotic group and 23 patients (82.1%) of the placebo group. There was a significant reduction of mild-to-moderate diarrhoea in the probiotic group compared with the placebo group ($p = 0.025$). Severe diarrhoea (grade 3 or 4) occurred only in five patients (17.9%) of the placebo group. There was no severe diarrhoea in the probiotic group. There was a significant reduction of severe diarrhoea in the probiotic group compared with the placebo group ($p = 0.05$). There was no grade 5 diarrhoea in this study.

Regarding the onset of diarrhoea, diarrhoea occurred at mean days of 20.5 (95% confidence interval 16.54–24.46) in the probiotic group and 17.44 (95% confidence interval 14.53–20.34) days in the placebo group. Probiotic group had a later onset of diarrhoea than the placebo group, but this was not statistically significant ($p = 0.321$). The comparison between cumulative incidences of the two groups was performed using Kaplan-Meier curves (see Fig. 3). Diarrhoea occurred after a mean dose of 29.43 Gy (95% confidence interval 23.76–35.1) in the probiotic group and 24.35 Gy (95% confidence interval 20.42–28.27) in the placebo group. Diarrhoea occurred after lower doses of radiotherapy in the placebo group compared to the probiotic group, but this was not statistically significant ($p = 0.159$). The comparison between the cumulative incidences of the two groups was performed using Kaplan-Meier curves (see Fig. 4).

The use of loperamide as an anti-diarrhoeal medication is shown in Fig. 2. In probiotic group, 13 patients (50%) had to use loperamide as an anti-diarrhoeal medication whereas in the placebo group, 24 patients (85.7%) used loperamide. Therefore, the use of loperamide as an anti-diarrhoeal medication was significantly lower in the probiotic group than the placebo group ($p = 0.005$). The mean time of loperamide use from the start of radiotherapy for probiotic group was 20.92 days with a 95% confidence interval between 16.75 and 25.1 and that of the placebo group was 18.04 days with a 95% confidence interval between 15.29 and 20.8. Loperamide was required earlier in patients taking placebo compared to those taking probiotic, but the association is not statistically significant ($p = 0.405$). The comparison between the two groups was performed using Kaplan-Meier curves (see Fig. 5). The mean dose of loperamide for probiotic group was 5.54 ± 5.04 mg and that of the placebo group was 8.08 ± 5.79 mg. Although higher doses of loperamide were required in the placebo group, there was no statistically significant difference between two groups ($p = 0.191$).

Grade 1 abdominal pain (according to CTCAE version 4.0) occurred in 19 patients (73.1%) in the probiotic group compared with 26 patients (92.9%) in the placebo group. Although grade 1 abdominal pain occurred more frequently in the placebo group, the association was not statistically significant and it narrowly missed the significance value ($p = 0.051$). Grade 2 abdominal pain occurred in 1 patient (3.8%) in the probiotic group compared with 16 patients (57.1%) in the placebo group. There was a very highly significant reduction of grade 2 abdominal pain in the probiotic group than the placebo group ($p = 0.000$). None of the patients in the probiotic group suffered from grade 3 abdominal pain, whereas three patients in the placebo group (10.7%) had it. However, the association is not statistically significant ($p = 0.086$). An episode of abdominal pain in days for the probiotic group was

Table 1 Baseline characteristics of the patients

Characteristics	Probiotic group ($n = 26$)Mean ± SD or number (%)	Placebo group ($n = 28$)Mean ± SD or number (%)	p
Age (years)	57.38 ± 10.75	52.5 ± 9.61	0.084^
History of surgery	4 (15.4%)	4 (14.3%)	0.910*
Chemotherapy	19 (73.1%)	22 (78.6%)	0.637*
Chemotherapy cycles (completed)	3.47 ± 1.65 ($n = 19$)	2.82 ± 1.14 ($n = 22$)	0.142^
ECOG status			0.145*
0	5 (19.2%)	11 (39.3%)	
1	17 (65.4%)	11 (39.3%)	
2	4 (15.4%)	6 (21.4%)	
Radiation dose (Gy)	50.77 ± 2.72	51.16 ± 3.43	0.646^
Duration of radiotherapy (days)	37.38 ± 6.49	37.21 ± 3.56	0.904^

*Chi-squared test

^Independent samples t test

3.63 ± 2.29 days and that of the placebo group was 7.77 ± 4.76 days. Since ($p = 0.000$), the reduction of the episode of abdominal pain in days in the probiotic group is very highly significant.

Interruption of radiotherapy due to diarrhoea is illustrated in Fig. 2. Only one patient (3.8%) in the probiotic group and three patients (10.7%) in the placebo group had treatment interruptions. Although the numerical data seemed different, there was no statistically significant difference between the two treatment groups ($p = 0.336$). During this study, there was no stopping of radiotherapy treatment owing to diarrhoea.

As the follow-ups, the patients were contacted at the 1 week, 2 weeks and 3 weeks after the completion of radiotherapy to assess the occurrence and grade of diarrhoea, loperamide use and grades of abdominal pain. Although the numerical data for these parameters were different between the probiotic and placebo groups, there were no statistically significant differences between the two groups.

Table 2 Stage and histological diagnosis of cervical cancer

	Probiotic group ($n = 26$)Number (%)	Placebo group (n=28)Number (%)
Stage of cervical cancer		
I B	2 (7.7%)	4 (14.3%)
II A	2 (7.7%)	1 (3.6%)
II B	12 (46.2%)	14 (50%)
III A	2 (7.7%)	4 (14.3%)
III B	7 (26.9%)	4 (14.3%)
IV A	1 (3.8%)	1 (3.6%)
Histological diagnosis		
Squamous cell carcinoma	23 (88.5%)	23 (82.1%)
Adenocarcinoma	2 (7.7%)	5 (17.9%)
Anaplastic carcinoma	1 (3.8%)	0 (0%)

ECOG performance status changes were noted at three times during the study: at the start and end of radiotherapy and 3 weeks after the radiotherapy. In general, the ECOG performance statuses increased from the baseline at the end of radiotherapy due to the side effects of radiotherapy including diarrhoea but these ECOG performance statuses decreased 3 weeks after the completion of treatment due to the recovery from such effects.

Discussion

A prophylactic treatment with NNT of 3.5 is rather promising, because probiotics have no serious side effects and the cost of treatment for a full course of about 37 days was approximately $US20. RID is troublesome and has long-term consequences. So, prevention is worthwhile. The probiotics could delay the onset of diarrhoea for about 3 days compared to the placebo, and it was 3 days later than the result of a study done at the same centre without any prophylaxis treatment [8]. Symptoms of RID were usually seen after an accumulated dose of 18–22 Gy during conventional fractionation [20]. In the current study, diarrhoea occurred after a mean dose of 29 Gy in the probiotic group. Probiotics could reduce all grades of abdominal pain and can significantly reduce the episode of abdominal pain in days. These may reflect the increased tolerance of the intestine to the higher doses of radiation and decreased in severity of radiation-induced enteritis.

Although the exact underlying mechanisms of using probiotics in RID were not yet clear, there were some proposed mechanisms. The possible mechanisms may be due to correction of dysbiosis. Probiotics lower the intestinal pH thereby setting the barrier to the potential pathogens. They may also cause down-modulation of the severity of intestinal inflammation by triggering and regulating the function of immune cells. The other mechanisms are down-modulation of

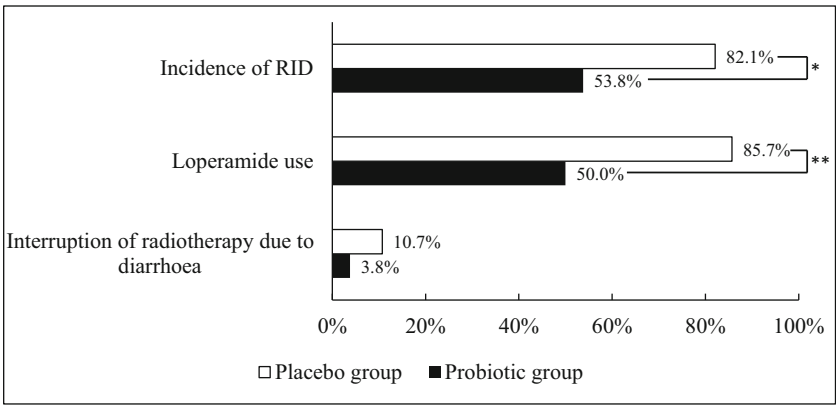

Fig. 2 Comparisons of the proportions of incidence of RID, loperamide use and interruption of radiotherapy due to diarrhoea between the probiotic group (n = 26) and the placebo group (n = 28)

*** = p < 0.05, ** = p < 0.01**

apoptosis which is regarded as the main factor responsible for the radiation-induced injury of the intestinal epithelium, up-regulation of the innate immune response in the gut which gives protection against intestinal colonization by invasive pathogens. They also stimulate lactase production, which helps lactose digestion because lactase may be reduced or loss due to the damage to the intestinal villi [15, 16, 21, 22].

The reduction of the interruptions of radiotherapy due to diarrhoea is also beneficial, because interruptions may lead to prolongation of total treatment time which in turn increases the risk of rapid repopulation of surviving tumour cells that is one of the most important factors controlling tumour response to fractionated radiotherapy [23]. Although the current study did not yield statistically significant differences in some variables, there was a trend in favour of using probiotics in reducing such variables. If the study is large and adequately powered, these variables may show a significant difference.

A placebo-controlled study done in 63 cervical cancer patients by Chitapanarux et al. [18] used probiotic capsules containing the same strain of *Lactobacillus* as current study but with a different strain of Bifidobacteria: *Bifidobacterium bifidum*. The dose was two billion colony-forming units (CFU) two times daily. The median age was not so different from the current study. They used Common Toxicity Criteria of NCI to assess the diarrhoea like the current study. In that study, the incidence of RID in both probiotic and placebo groups was 100%. This may be due to the fact that all patients received weekly cisplatin which may aggravate diarrhoea. In

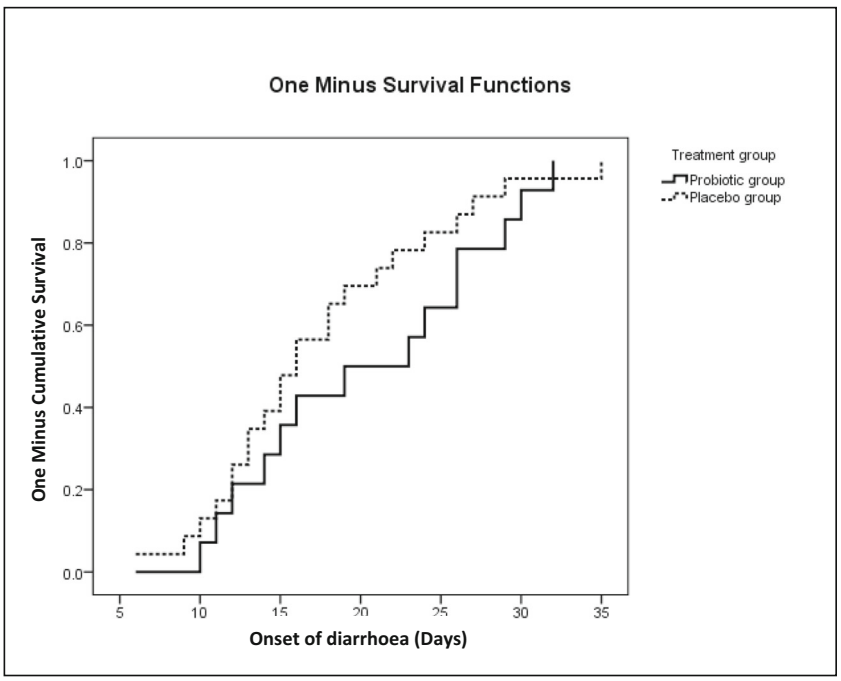

Fig. 3 Kaplan-Meier curves showing onset of diarrhoea by days of radiotherapy in probiotic and placebo groups

***p* = 0.321** by Log Rank (Mantel-Cox) test

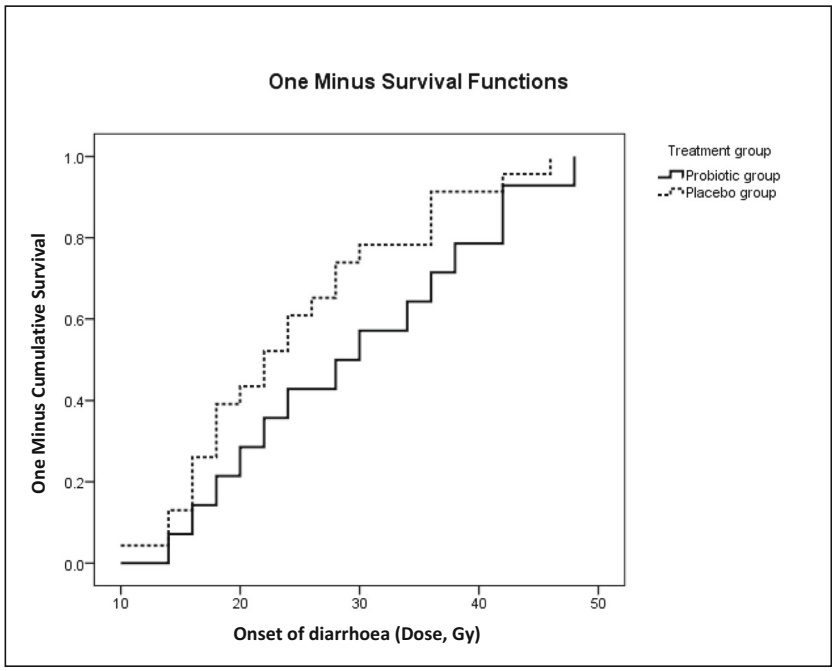

Fig. 4 Kaplan-Meier curves showing onset of diarrhoea by dose (Gy) of radiotherapy in probiotic and placebo groups

p = **0.159** by Log Rank (Mantel-Cox) test

the current study, only about 70% of the patients in both probiotic and placebo group received chemotherapy. There was a significant reduction of grade 2 and grade 3 diarrhoea in the probiotic group. The 32% of the patients in the placebo group needed anti-diarrhoeal medication-loperamide vs. 9% of the patients in the probiotic group. It is similar to the findings of the current study, but the percentages are much lower [18]. The other double-blind placebo-controlled study done in 490 cervical, sigmoid or rectal cancer patients by Delia et al. [17] used a probiotic powder which contains four strains of lactobacilli (*Lactobacillus casei*, *Lactobacillus plantarum*, *L. acidophilus* and *Lactobacillus delbrueckii* subsp.

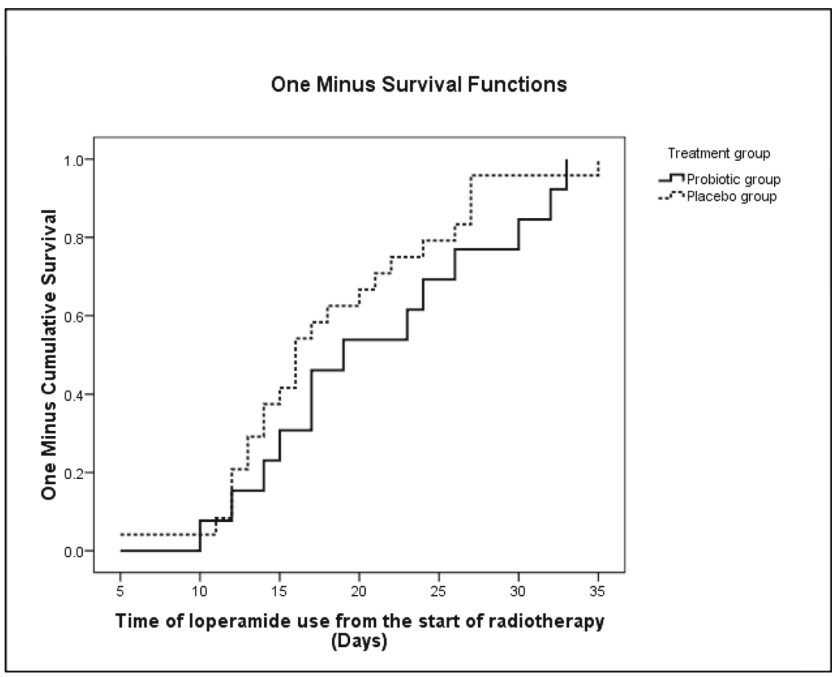

Fig. 5 Kaplan-Meier curves showing time of loperamide use from the start of radiotherapy (Days) in probiotic and placebo groups

p = **0.405** by Log Rank (Mantel-Cox) test

bulgaricus) and three strains of Bifidobacteria (*Bifidobacterium longum*, *Bifidobacterium breve* and *Bifidobacterium infantis*) with the dose of 450 billion bacteria, three times per day. More patients in the placebo group had RID compared with the probiotic group (51.8 vs. 31.6%) achieving ARR of about 20% and NNT was about five. Although many strains of probiotics at significantly higher doses were used, those results are not so different from the current study. In that study, probiotics can significantly reduce the grade 3 and grade 4 of the WHO diarrhoea grading. In comparing the studies, there are some difficulties because of the differences in the diarrhoea grading systems. Some used Common Toxicity Criteria of NCI as the current study [18], and others used WHO grading [16, 17]. The mean time of loperamide use from the start of radiotherapy for probiotic group was 122 h (5.08 days) and that of the placebo group was 86 h (3.58 days), and these are earlier than those from the current study. This may be due to the recruitment of colorectal cancer patients in whom the change in the bowel habit is more common [17]. Another double-blind placebo-controlled study done by Giralt et al. [22] used the probiotic liquid yogurt containing *L. casei* DN-114001 at 9.6 billion CFU there times daily. A total of 85 patients with cervical cancer (radiotherapy and weekly cisplatin) or endometrial adenocarcinoma (post-operative radiotherapy) were recruited. There was the modest reduction of the incidence of RID with ARR of 3.45% and NNT was 39. There was an increase in the proportion of patients who required loperamide in the probiotic group (36.36%) compared with the placebo group (29.27%). These findings may be due to the using of only one strain of probiotics although in a larger dose [24]. Another study done by Demers et al. [16] used probiotic *L. acidophilus* LAC-361 and *B. longum* BB-536 in two different dosage regimens: a standard dose of 1.3 billion CFU twice a day or a high dose of 10 billion CFU three times a day. Two hundred twenty-nine patients with gynaecologic, rectal or prostate were included. WHO diarrhoea grades, NCI abdominal pain grades and daily number of bowel movements did not differ significantly between the probiotic and the placebo group in both dosage regimens. The percentage of patients who took loperamide was 42.5, 30.2 and 27.4% for placebo, standard-dose probiotic and high-dose probiotic groups, but the difference between these groups was not significant. In that study, the first capsule of loperamide was taken on day 19.7 (placebo), 20.4 (standard dose of probiotics) and 20.9 (high dose of probiotics). Those results were similar to the current study. Treatment interruptions did not differ significantly as the current study [16].

Lactobacillus and Bifidobacteria are the most commonly used strains in the studies done for the RID [14]. Delia et al. suggested the use of several selected strains may enhance the competition between the probiotic organisms and intestinal flora and the synergistic effect of combining more than one strain may greatly enhance the suppression of potential pathogens [17]. The total dose used by the current study was a little more than the other two studies which used the approximately the same strains and three times daily dosing may help the continuous supply of probiotics to the intestine [16, 18]. The current study used probiotic capsules which are the most commonly used dosage form for probiotics, because they have some advantages such as higher potency and longer stability [25]. The capsules were the hard shell gelatin capsules which were not acid resistant, but the strains used in the current study survived well in the human gastro-intestinal tract [26–28].

Diagnosing RID was one of the limitations of current study, because there was no specific quantitative biomarker to diagnose and assess the degree of radiation enteritis. Therefore, the diagnosis of acute radiation enteritis has to be made presumptively based on symptoms and their onset [6]. Some of the markers such as acute phase response-related markers, fatty acids, vitamin E, leucocyte count, haemoglobin, citrulline and leukotriene B4 can be measured in blood, lactoferrin and calprotectin in stool and fatty acids in rectal mucosal biopsies [10, 29]. But their time courses vary, and they are not specific and some of them are not in common use. Although endoscopic biopsies may reveal histologic changes consistent with radiation damage, it was not feasible in the current study. Therefore, patients who had fever during diarrhoea episode with blood and mucous in the stool were excluded to rule out the infective causes of diarrhoea but stool cultures were not performed. Chitapanarux et al. perform the white blood cell count and red blood cell count in the stool instead of the stool culture [18]. However, these cell counts are not specific to radiation enteritis and they can also be found in any inflammation or infections. To control the previous confounding factors, this study was designed to incorporate randomization, restriction and matching. Some studies used assessment of stool consistency as an end point [16, 18, 24, 30]. Stool consistency assessment was considered during the study planning phase but rejected due to the potential difficulty to get the reliable information from the patients. The adherence to the standard dietary recommendations could only be checked by the investigator and not by the registered dietician.

Some studies started the probiotic treatment prior to the radiotherapy for the early colonization of the intestine with probiotic bacteria producing mixed results [18, 24]. Studies done by Delia et al., Timko and Demers et al. started the treatment on the first day of radiotherapy, and the former gained positive results in 490 patients [16, 17, 19]. So, in the current study, starting the treatment on the first day of radiotherapy (well before the onset of RID) was chosen and also for the feasibility.

RID has not occurred in all patients receiving pelvis radiotherapy without any prophylactic treatments. And from the current study, 17.9% of the patients in the placebo group had no RID. Although there might be some extent of the placebo effect, the host and microbial genetic differences may

influence on the radiosensitivity of the intestine. There were emerging but not matured data supporting this. Iwata et al. [31] identified a correlation between single-nucleotide polymorphisms (SNPs) in mice and the amount of jejunal crypt cell apoptosis after 2.5 Gy whole body irradiation. Genome-wide analysis found that radiation-induced jejunal apoptosis was associated with eight SNP markers, suggesting radiosensitivity of intestine may vary with genetic variations [31]. A study done by the Manichanh et al. [32] used DNA fingerprinting to identify the individuals' microbial profiles and found that the initial intestinal microbial composition of each individual could be a determinant for developing postirradiation diarrhoea. Intestinal microbial compositions of patients with diarrhoea changed markedly during and 7 week after radiotherapy. That study also indicated that the patients' initial microbial composition before radiotherapy may determine the occurrence of RID [32]. These will be the future directions of the studies done for RID.

Conclusions

Probiotics could significantly reduce the incidence and severity grades of RID, the severity and episode of abdominal pain (in days) and the use of anti-diarrhoeal drug—loperamide. But they could not significantly reduce the dose of loperamide and could not prevent the interruption of radiotherapy due to diarrhoea. It also could not significantly slow down the onset of diarrhoea and onset of the use of loperamide. Probiotics had no statistically significant effects on the patients' conditions during the 3 weeks follow-up and ECOG performance status changes. As the probiotics achieved significant absolute risk reduction (ARR) and number needed to treat (NNT) 3.5, its supplementation is thought to be worthwhile in clinical practice. Therefore, from this study, it can be concluded that the supplementation of probiotics containing live *L. acidophilus* LA-5 plus *B. animalis* subsp. *lactis* BB-12 is an easy and effective way to prevent the acute RID among cervical cancer patients.

Acknowledgements We wish to thank Prof. Khin Cho Win, Prof. Khin Mar Myint, Prof. Nu Nu Aye, Dr. Ngu Wah Than, Dr. Khin Maung Lwin and Ms. Lwin Mon Mon Myint for their kind help and advices.

Compliance with Ethical Standards

This study was approved and passed by the Postgraduate Board of Studies (Pharmacology), University of Medicine 1, Yangon (which is an institutional review board (IRB)), and this trial was registered at the Thai Clinical Trial registry with the trial registration number TCTR20170314001. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. World Health Organization (WHO) (2015) World cancer report, 2014. http://www.who.int/mediacentre/factsheets/fs297/en/. Accessed 2 Nov 2016
2. ICO Information Centre on HPV and Cancer (2016) Myanmar human papillomavirus and related cancers, Fact Sheet 2016 (October 7, 2016). http://www.hpvcentre.net. Accessed 20 Oct 2016
3. Department of Radiotherapy of Yangon General Hospital (2017) Leading cause of morbidity by sex. Yangon General Hospital from 01/01/2016 to 31/12/2016
4. Walsh D (1987) Deep tissue traumatism from roentgen ray exposure. Br Med J 2:272–273
5. McGough C, Baldwin C, Frost G, Andreyev NJH (2004) Role of nutritional intervention in patients treated with radiotherapy for pelvic malignancy. Br J Cancer 90:2278–2287
6. Packey CD, Ciorba MA (2010) Microbial influences on the small intestinal response to radiation injury. Curr Opin Gastroenterol 26: 88–94
7. Visich KL, Yoe TP (2010) The prophylactic use of probiotics in the prevention of radiation therapy-induced diarrhea. Clin J Oncol Nurs 14:467–473
8. Moe-Moe-Thet-Win (2006) A clinical study of common acute radiation morbidity of cervical cancer patients undergoing pelvis radiation at Radiation Oncology Department of Yangon General Hospital. Dissertation, University of Medicine 1, Yangon :44–45
9. Picard C, Wysocki J, Fioramonti J, Griffiths NM (2001) Intestinal and colonic motor alterations associated with irradiation-induced diarrhoea in rats. Neurogastroenterol Motil 13:19–26
10. Larsen A, Bjørge B, Klementsen B, Helgeland L, Wentzel-Larsen T, Fagerhol MK (2007) Time patterns of changes in biomarkers, symptoms and histopathology during pelvic radiotherapy. Acta Oncol 46:639–650
11. Donaldson S, Jundt S, Ricour C, Sarrazin D, Lemerie J, Schweisguth O (1975) Radiation enteritis in children. A retrospective review, clinicopathologic correlation and dietary management. Cancer 35:1167–1178
12. Gibson RJ, Keefe DM, Lalla RV, Bateman E, Blijlevens N, Fijlstra M (2013) Systematic review of agents for the management of gastrointestinal mucositis in cancer patients. Support Care Cancer 21: 313–326
13. Food and Agricultural Organization (FAO)/World Health Organization (WHO) (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. FAO/WHO, Córdoba, p 6
14. Hamad A, Fragkos KC, Forbes A (2013) A systematic review and meta-analysis of probiotics for the management of radiation induced bowel disease. Clin Nutr 32:353–360
15. Paris F, Fuks Z, Kang A, Capodieci P, Juan G, Ehleiter D, Haimovitz-Friedman A, Cordon-Cardo C, Kolesnick R (2001) Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science 293:293–297
16. Demers M, Dagnault A, Desjardins J (2014) A randomized double-blind controlled trial: impact of probiotics on diarrhea in patients treated with pelvic radiation. Clin Nutr 33:761–767

17. Delia P, Sansotta G, Donato V, Frosina P, Messina G, Renzis CD (2007) Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol 13:912–915
18. Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, Lorvidhaya V (2010) Randomized controlled trial of live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. Radiat Oncol 5(31):1–6
19. Timko J (2010) Probiotics as prevention of radiation-induced diarrhoea. J Radiother Pract 9:201–208
20. Hopkins M (2004) Radiation induced diarrhoea—literature review. Radiographer 51(1):41–45
21. Blanarova C, Galovicova A, Petrasova D (2009) Use of probiotics for prevention of radiation-induced diarrhoea. Bratisl Lek Listy 110(2):98–104
22. Liu MM, Li ST, Shu Y, Zhan HQ (2017) Probiotics for prevention of radiation-induced diarrhea: a meta-analysis of randomized controlled trials. PLoS ONE 12(6):e0178870. https://doi.org/10.1371/journal.pone.0178870 Accessed 25 Dec 2017
23. Barrett A, Dobbs J, Morris S, Roques T (2009) Practical radiotherapy planning, 4th edn. Hodder Arnold, Italy, pp 39–40
24. Giralt J, Regadera JP, Verges R, Romero J, Fuente IDL, Biete A, Villoria J, Cobo JM, Guarner F (2008) Effects of probiotic *Lactobacillus casei* DN-114 001 in prevention of radiation-induced diarrhoea: results from multicenter, randomized, placebo-controlled nutritional trial. Int J Radiat Oncol Biol Phys 71(4): 1213–1219
25. RodriguezP (2015) Probiotics: 3 dosage forms to consider for a great product. http://knowledge.ulprospector.com/2198/fbn-probiotics-3-dosage-forms/. Accessed 25 Nov 2016
26. Alander M, Matto J, Kneifel W, Johansson M, Kogler B, Crittenden R, Mattila-Sandholm T, Saarela M (2001) Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of *Bifidobacterium lactis* Bb-12 in the gastrointestinal tract. Int Dairy J 11(10):817–825
27. Matto J, Fonde'n R, Tolvanen T, Wright AV, Vilpponen-Salmelad T, Satokari R, Saarela M (2006) Intestinal survival and persistence of probiotic *Lactobacillus* and *Bifidobacterium* strains administered in triple-strain yoghurt. Int Dairy J 16(10):1174–1180
28. Savard P, Lamarche B, Paradis ME, Thiboutot H, Laurin E, Roy D (2011) Impact of *Bifidobacterium animalis subsp. lactis* BB-12 and, *Lactobacillus acidophilus* LA-5-containing yoghurt, on fecal bacterial counts of healthy adults. Int J Food Microbiol 149:50–57
29. Lutgens LCHW, Deutz N, Granzier-Peeters M, Beets-Tan R, Ruysscher DD, Gueulette J, Cleutjens J, Berger M, Wouters B, Meyenfeldt MV, Lambin P (2004) Plasma citrulline concentration: a surrogate end point for radiation-induced mucosal atrophy of the small bowel. A feasibility study in 23 patients. Int J Radiat Oncol Biol Phys 60(1):275–285
30. Urbancsek H, Kazar T, Mezes I, Neumann K (2001) Results of a double-blind, randomized study to evaluate the efficacy and safety of Antibiophilus® in patients with radiation-induced diarrhoea. Eur J Gastroenterol Hepatol 13:391–396
31. Iwata M, Iwakawa M, Noda S, Ohta T, Minfu Y, Kimura T, Shibuya H, Imai T (2007) Correlation between single nucleotide polymorphisms and jejunal crypt cell apoptosis after whole body irradiation. Int J Radiat Biol 83(3):181–186
32. Manichanh C, Varela E, Martinez C (2008) The gut microbiota predispose to the pathophysiology of acute post-radiotherapy diarrhea. Am J Gastroenterol 103:1754–1761