Improving Menstrual Health Throughout the Reproductive Life Course – Original Research Article

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The role of probiotics in improving menstrual health in women with primary dysmenorrhoea: A randomized, double-blind, placebo-controlled trial (the PERIOD study)

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Abstract

Background: Primary dysmenorrhea is associated with poorer quality of life; however, the causal mechanism remains unclear. A vast body of literature supports the use of oral probiotics for relief from the symptoms of endometriosis; however, to our knowledge, no study has prescribed probiotics for primary dysmenorrhea.

Objective: The aim of this study is to investigate the effects of 3-month supplementation with oral probiotics on quality of life and inflammatory markers in women with primary dysmenorrhea.

Design: Randomized placebo-controlled trial.

Methods: A total of 72 patients (36 patients in each arm) were randomized to receive either oral sachets containing 5 billion colony-forming units each of *Lactobacillus acidophilus BCMC (BCrobes Microbial Cells) 12130, Lactobacillus casei subsp BCMC 12313, Lactobacillus lactis BCMC 12451, Bifidobacterium bifidum BCMC 02290, Bifidobacterium longum BCMC 02120, and Bifidobacterium infantis BCMC 02129* each or placebo twice daily for 3 months. Main outcome measures were visual analog scale, verbal rating scale, physical and mental health scores using Short-Form 12-Item version 2 questionnaire, frequency of nonsteroidal anti-inflammatory drug use, and changes in inflammatory markers (interleukin-6, interleukin-8, and tumor necrosis factor alpha) before and after treatment.

Results: There was no significant difference in the quality of life scores between the probiotic and placebo groups. Both groups showed significant improvement in pain (visual analog scale) and severity (verbal rating scale) scores but the probiotic group had much lower nonsteroidal anti-inflammatory drug use (odds ratio: 0.69, 95% confidence interval: 0.26–1.83) and better mental health scores (mean change: 6.5, $p = 0.03$ versus 6.1, $p = 0.08$) than the placebo group. There was a significant confounding effect of nonsteroidal anti-inflammatory drug use on quality of life scores. No significant difference was found in inflammatory cytokines.

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Conclusion: Tested oral probiotics improved mental health and potentially reduced the use of nonsteroidal anti-inflammatory drugs; however, there was no significant change in inflammatory markers. Further research with a larger sample size is needed to confirm the findings.
Registration: This study is registered under ClinicalTrials.gov (NCT04119011).

Plain Language Summary
Use of Probiotic in Primary Dysmenorrhoea
This study looked at whether taking probiotics (good bacteria) for 3 months could improve the quality of life and reduce pain in women with painful periods. The study found that probiotics did not significantly improve quality of life scores, but did reduce the use of painkillers and improve mental health scores. However, the probiotics did not have a significant effect on inflammatory markers in the body. More research is needed to confirm the findings.

Keywords
inflammatory markers, menstrual disorders, primary dysmenorrheal, probiotics, quality of life

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Introduction

Primary dysmenorrhea is a common problem faced by women of reproductive age and is often underappreciated. Women with dysmenorrhea often have limited work and school functioning. It is a major cause of absenteeism and reduced quality of life.[1–3] Young women with dysmenorrhea are also at risk of chronic pain later in life owing to central sensitization.[4] Primary dysmenorrhea affects women with regular menstrual cycles in the absence of organic diseases such as endometriosis, uterine leiomyoma, adenomyosis, or other uterine or ovarian pathologies.[5,6] However, the exact pathogenesis of primary dysmenorrhea remains unknown. Many studies have attributed pain, uterine contraction, and inflammation to prostaglandins (PGs).[7] The intensity of menstrual cramps and associated symptoms of dysmenorrhea are directly proportional to the amount of PGF2α released.[8] The synthesis of PGF2α (Prostaglandin F 2 alpha) is proven to be upregulated by proinflammatory cytokines, such as interleukins (IL)-6 and IL-8, and downregulated by anti-inflammatory markers like IL-11.[9] Leukotrienes, another by-product of the lipoxygenase enzyme pathway, also exacerbate uterine contractions; however, their actions are not affected by nonsteroidal anti-inflammatory drugs (NSAIDs).[10] This may explain why NSAIDs are not effective in some patients with primary dysmenorrhea. The effect of other mediators, such as progesterone, vasopressin, and calcium channels, has also been examined in some studies; however, evidence regarding the usefulness of their therapeutic action is still lacking.[11]

The conventional treatment for primary dysmenorrhea relies on the use of oral NSAIDs and hormonal pills to reduce inflammation and suppress ovulation. However, these treatments have been found to have adverse effects on health and cancer risk, particularly with long-term use. NSAIDs can lead to organ damage via the build-up of oxidative stress species, while oral contraceptive pill users have an increased risk of breast cancer, as reported in recent literature.[12,13] Furthermore, some patients fail to respond to NSAIDs, and hormonal therapy is contraindicated in some cases; therefore, limited treatment options are available.[14] The search for an alternative treatment with few side effects, especially for long-term use, is still ongoing.

Probiotics can be used as an alternative treatment option for primary dysmenorrhea. Probiotic supplements have been shown to colonize the human intestine and confer many health benefits, particularly in immunomodulatory and inflammatory conditions.[15] Probiotics are live microorganisms that have beneficial health effects in many disease states. Certain metabolic processes are regulated through diverse human microbiomes that promote favorable conditions in the host's intestinal environment.[16] In patients with endometriosis, the use of *Lactobacillus gasseri GG* has been found to suppress the development of ectopic endometriotic lesions in a murine model and lead to improved visual analog scale (VAS) and verbal rating scores for dysmenorrhea.[17] Khodaverdi et al.[18] reported improved pain scores after the use of oral *Lactobacillus* supplements in patients with endometriosis.

However, studies on the use of probiotics in women with primary dysmenorrhea are limited. Hence, we designed this study to examine the potential benefits of probiotics as a novel treatment for women with primary dysmenorrhea. We hypothesized that oral supplementation with probiotics for 3 months will restore gut dysbiosis and reduce inflammatory cytokines, thus improving the quality of life in women with primary dysmenorrhea.

Materials and methods

Participants

A sample size of 30 was calculated based on a study by Safdari and Parvin to detect a mean score difference of

0.92 in the VAS scores between the probiotic and placebo groups, with a power of 80% and a two-sided type-1 error of 0.05.[19] Considering a dropout rate of 20%, the final sample size of 72, with 36 participants in each arm, was determined. Due to inadequate funding, only 28 samples were analyzed for the analysis of inflammatory markers. The pretreatment and post-treatment samples were randomly selected from each treatment group (eight from the probiotic group and six from the placebo group).

Women who presented with primary dysmenorrhea at gynecology clinics or wards of the University of Kebangsaan Malaysia Medical Center (UKMMC) were eligible to participate in the study. All the participants provided written informed consent. Premenopausal women with a regular menstrual cycle, primary dysmenorrhea, and compliant with oral sachets consumed twice daily for 3 months were eligible. Primary dysmenorrhea was diagnosed according to the history and medical examination data (recurrent menstrual pain on the first 2 days of menstruation without identifiable pathology), confirmed by pelvic ultrasonography when the diagnosis was doubtful. Women on intrauterine copper devices, with a history of hormonal treatment or contraceptives within the past 3 months, who had food allergies or were lactose intolerance, who had suspected or confirmed tumor or malignancy, who frequently used laxative or antidiarrheal drugs, or who were receiving treatment for allergic diseases were excluded.

Recruitment

The researchers identified eligible participants from among the patients at the clinic and/or ward. Women who agreed to participate and met the inclusion criteria were counseled regarding the study, and written consent was obtained. Seventy-eight potential candidates were identified between October 2019 and December 2019. Six women were excluded because they were unwilling to participate or did not meet the inclusion criteria. A total of 72 women were randomized to placebo and probiotic groups. At the end of the study, five participants were lost to follow-up. The final analysis included 67 (93%) of the randomized women, as shown in Figure 1. This study adhered to the CONSORT checklist.

Study procedure

This double-blind, randomized, placebo-controlled trial was conducted at the UKMMC between October 2019 and March 2020. Upon recruitment, the participants' demographic data and menstrual history were collected. The participants were asked to complete the VAS, verbal rating scale (VRS), and Short-Form 12-item version 2 (SF12v2) questionnaires on the first 2 days of their menses. Blood samples were collected on day 2 of menstruation in a 5-mL plain tube. The samples were centrifuged at 4000 r for 20 min to separate the serum that was subsequently stored at −80°C until further processing for inflammatory markers. All the participants were given a pain diary to document menstrual flow, pain intensity, and frequency of oral NSAID use throughout the 3-month treatment period. The use of analgesics other than NSAIDs was not assessed. The patients were also asked to maintain a pain diary.

The participants were randomized upon recruitment using a computerized randomization sequence in a 1:1 ratio, and designated A or B, and treatments were dispensed accordingly. Probiotic and placebo sachets were prepared by a probiotic-manufacturing company using identical packaging. Each sachet was individually labeled as A or B and contained either probiotics or placebo. The sachets were then packaged into identical tamper-proof boxes with details regarding the expiry date, storage instructions, and instructions for use. Only the manufacturer was aware of the contents of the sachets. Both the researchers and participants were blinded to the study grouping until data collection was completed.

The treatment group received oral probiotic sachets containing 5 billion colony-forming units each of *Lactobacillus acidophilus BCMC 12130, Lactobacillus casei subsp BCMC 12313, Lactobacillus lactis BCMC 12451, Bifidobacterium bifidum BCMC 02290, Bifidobacterium longum BCMC 02120*, and *Bifidobacterium infantis BCMC 02129*, while the placebo group received identical sachets containing excipients, namely maltodextrin, lactose, oligosaccharides, citric acid, ascorbic acids, and flavoring agents (B-Crobes, Subang Jaya, Malaysia). The participants were instructed to consume one sachet twice daily with meals for 3 months. The participants received 250 mg of oral mefenamic acid (Ponstan) for use when needed during the study period.

The participants were contacted once a month, either by face-to-face consultation or via mobile phone, to assess and emphasize compliance and monitor side effects. They were deemed compliant if they consumed more than 80% of treatment sachets, as determined by counting the remaining sachets at each visit. At the end of the third month of treatment, the participants were asked to assess the first 2 days of their menses, whereby the VAS, VRS, and SF12v2 questionnaires and blood collection were repeated. The patients were also checked.

Outcomes

The primary outcome was improvement in quality of life, reflected by the pain, severity, physical health, and mental health scores and frequency of NSAID use on the second day of menstruation before and after treatment. The pain score was measured using the 10-point VAS and severity score, using the VRS (grades 0–3).[20–22] The SF12v2 questionnaire assessed the impact of cyclical menstrual pain on

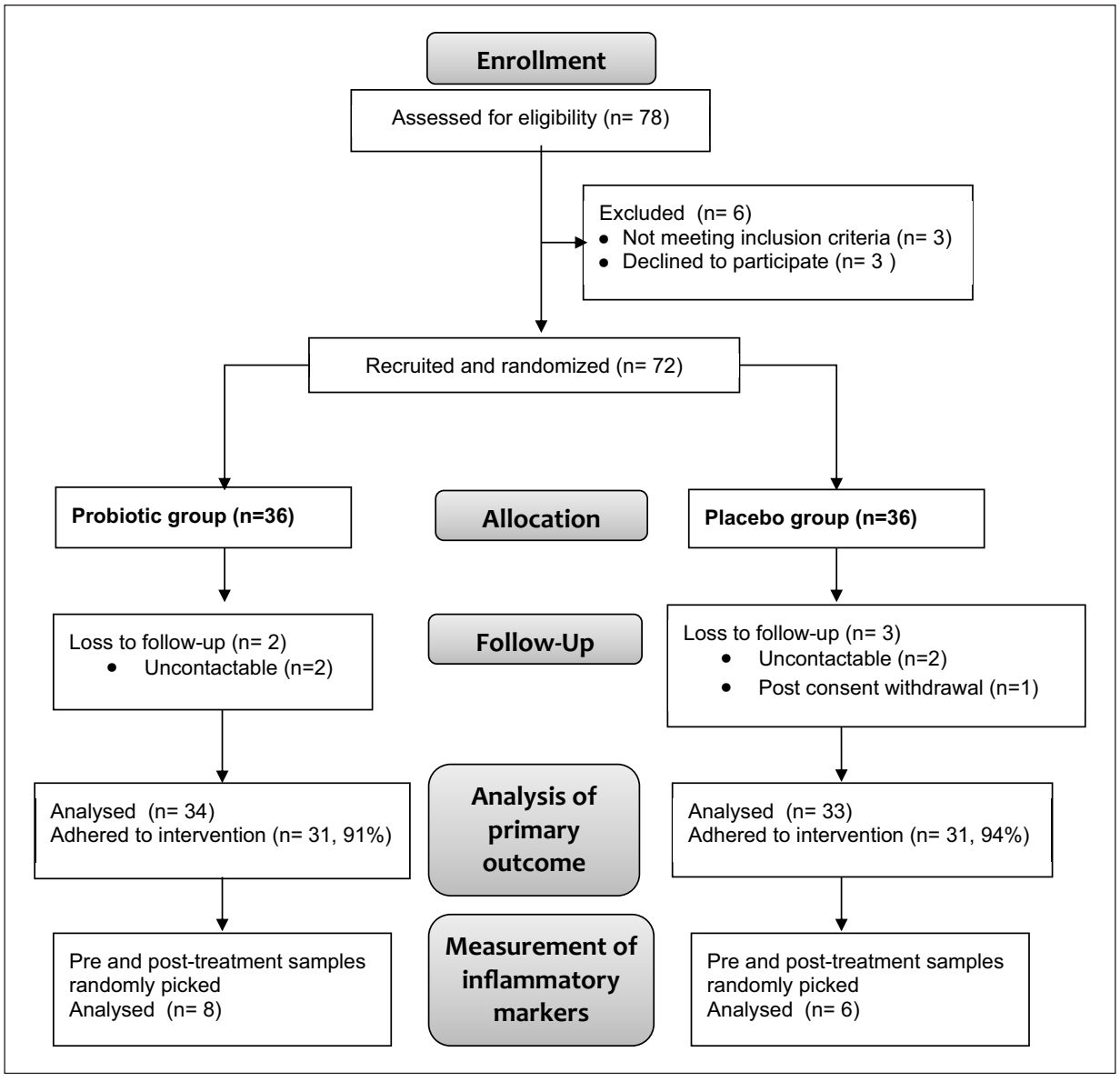

Figure 1. Flowchart showing numbers of participants at each stage of the trial.

physical and mental health, where the score was transformed into a score out of 100% according to the given formula.[20–22] Permission for the use of this questionnaire was obtained from the primary author.

The secondary outcome was the difference in the concentrations of inflammatory markers before and after treatment. The levels of inflammatory markers IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) were measured using the multiplex immunoassay MILLIPLEX MAP Human High Sensitivity T-Cell Magnetic Bead Panel (Prima Nexus, Kuala Lumpur, Malaysia). Each sample was incubated with cytokine antibodies, washed, and analyzed using a Magpix reader (Luminex Corp., Austin, TX, USA). The fluorescence unit was then converted to a cytokine concentration unit to obtain the measurement levels. In 2013, Ma et al.[9] investigated the changes in inflammatory cytokines during menstrual cycles in a small number of participants (six with dysmenorrhea and three controls). Similarly, owing to limited funding, only 28 samples were randomly selected and analyzed in our study (eight and six pretreatment and post-treatment samples from the probiotic and placebo groups, respectively).

Statistical analysis

All analyses were performed using the Statistical Package for Social Sciences (SPSS), version 23. Descriptive analysis was used to report the baseline characteristics of the

Table 1. Baseline characteristics of participants.

Characteristics	Probiotic ($n=36$)	Placebo ($n=36$)	p
Participant characteristics			
Age (years), median (IQR)	25 (5)	26 (7)	0.564
Menarche (years), median (IQR)	12 (2)	12 (1)	0.730
Menstrual cycle (days), median (IQR)	30 (4)	30 (6)	0.895
Menstrual flow (days), median (IQR)	7 (1)	7 (3)	0.275
Education level, *n* (%)			
Tertiary	35 (97.2)	34 (94.4)	1.000
Postgraduate	1 (2.8)	2 (5.6)	
Marital status, *n* (%)			
Single	29 (80.6)	30 (83.3)	1.000
Married	7 (19.4)	6 (16.7)	
Ethnicity, *n* (%)			
Malay	28 (77.8)	31 (86.1)	0.234
Chinese	5 (13.9)	4 (11.1)	
Indian	3 (8.3)	0 (0.0)	
Others	0 (0.0)	1 (2.8)	
Occupation, *n* (%)			
Unemployed	17 (47.2)	15 (42.9)	0.174
Employed	14 (38.9)	18 (51.4)	
Self-employed	1 (2.8)	2 (5.7)	
Others	4 (11.1)	1 (2.8)	

IQR: interquartile range.

study participants according to the treatment group. Continuous variables were reported as means and standard deviations, whereas categorical or ordinal variables were reported as absolute and relative frequencies. For outcome measurements, the analysis was based on intention-to-treat, and participants with incomplete data were excluded. We calculated the mean scores for each life parameter and conducted an independent samples *t*-test to compare normally distributed data between the probiotic and placebo groups. We also used paired *t*-tests to compare baseline and post-treatment values within groups. A multivariate binary logistic regression model was run to assess the effect of potential confounders, such as the frequency of NSAID use and VAS, VRS, and SF12v2 scores that were included in the model as covariates for the treatment group. The results were further stratified according to confounding factors. Statistical significance was set at $p < 0.05$.

Results

Study demographics

The characteristics of the participants in the probiotic and placebo groups were similar (Table 1). Most women were single and had completed tertiary-level education. The age range of the participants was 23–41 years. The distribution of ethnic backgrounds was similar between the groups. The menstrual patterns were also similar in both groups. A large proportion of the study participants was unemployed and comprised students.

Quality of life parameters

Before treatment, the quality of life scores in both groups was comparable. Pain score and severity were reported as moderate, while physical and mental health scores were above 50% (Table 2).

After 3 months of treatment, the probiotic group showed a significant improvement in mental health scores before and after treatment (mean change: 6.5, $p=0.03$) compared with the placebo group (mean change: 6.1, $p=0.08$). Both groups showed significant improvements in the VAS and VRS scores. The placebo group showed significantly lower VRS scores than the probiotic group. No other significant differences were found in other quality-of-life parameters.

The frequency of NSAID use was lower in the probiotic group (55.9%) than in the placebo group (64.7%); however, the difference was not statistically significant (Table 2). The odds ratio of using NSAIDs was 0.69 times lower for the probiotic group than for the placebo group (95% confidence interval: 0.26–1.83). Multivariate binary logistic regression analysis controlling for potential confounders, that is, frequency of NSAID use and VAS, VRS, and SF12v2 scores, demonstrated a significant association between the frequency of NSAID use and the severity score (VRS) (Table 3).

Table 2. Quality of life scores before and after treatment according to the treatment group ($n = 67$).

Quality of life	Mean (*SD*)	Probiotic ($n = 34$)	Placebo ($n = 33$)	p[a]
Pain score (VAS)	Baseline	6.6 (1.7)	6.4 (1.7)	
	After treatment	4.0 (2.2)	3.9 (2.6)	0.69
	Changes	**2.6**[b] (2.5)	**2.5**[b] (3.1)	
Severity score (VRS)	Baseline	1.8 (0.7)	1.9 (0.7)	
	After treatment	1.3 (0.6)	1.1 (0.7)	**0.03**
	Changes	**0.5**[b] (0.8)	**0.8**[b] (0.9)	
Physical health score (SF12v2)	Baseline	73.1 (16.9)	73.8 (14.0)	
	After treatment	77.6 (13.4)	80.8 (18.4)	0.65
	Changes	4.5 (19.1)	6.9 (21.0)	
Mental health score (SF12v2)	Baseline	68.2 (13.2)	69.2 (15.4)	
	After treatment	74.7 (13.4)	75.4 (19.2)	0.74
	Changes	**6.5**[b] (19.1)	6.1 (19.3)	
Frequency of NSAID use (number of tablets)	Total	2.4 (3.6)	4.1 (6.6)	0.21
	Average per cycle	0.8 (1.2)	1.3 (2.2)	0.28

SD: standard deviation; VAS: visual analog scale; VRS: verbal rating scale; SF12v2: Short-Form 12-item version 2; NSAID: nonsteroidal anti-inflammatory drug.
[a]Data were analyzed by independent *t*-test to determine differences between treatment groups.
[b]$p < 0.05$, paired *t*-test analysis within the treatment group.

Table 3. Multivariate logistic binary regression analysis of potential confounders showing the association between VRS score and frequency of NSAID use.

Co-variates	AOR	95% CI	p
Difference in VAS	0.776	0.58–1.03	0.23
Difference in physical health score	0.973	0.93–1.02	0.53
Difference in mental health score	0.983	0.93–1.04	0.08
Frequency of NSAID use	1.39	1.11–1.73	**0.004**

AOR: adjusted odd ratio; CI: confidence interval; NSAID: nonsteroidal anti-inflammatory drug; VAS: visual analog scale.

Stratification analysis

The results were further stratified according to NSAID use. The placebo group showed significantly better VRS scores when NSAIDs were administered. No significant differences were noted between the groups with respect to other parameters. In addition, the significance of improvement in mental health scores in the probiotic group was lost after adjusting for NSAID use (Table 4).

Inflammatory markers

No significant difference was found in the levels of inflammatory markers before and after treatment in either group; however, the sample size for this analysis was small owing to inadequate funding (Figure 2).

Side effects of treatment

A few side effects such as diarrhea, bloating, and fever were reported in the placebo group ($n = 3$) but no side effects were reported in the probiotic group.

Discussion

The aim of this trial was to assess the effects of a 3-month oral probiotic supplementation in women with primary dysmenorrhea compared with placebo on the quality of life and the effect on inflammatory cytokines on the same. Multiple tools were used to assess the quality of life, including subjective (VAS, VRS, and SF12v2 questionnaires) and objective (frequency of NSAID use) measures. Regarding inflammatory cytokines, we chose IL-6, IL-8, and TNF-α, since their correlations with primary dysmenorrhea are well established in the literature.[9,11]

Oral supplementation with the tested probiotics for women with primary dysmenorrhea did not significantly improve their quality of life or inflammatory markers compared with placebo. However, there was no significant reduction in NSAID use in the probiotic group either. Long-term NSAID use is associated with side effects, such as renal impairment and peptic ulcer disease; hence, reduced dependence on NSAIDs could help reduce the risk of side effects, thus improving the quality of life.

The most likely explanation for the lack of differences found between the probiotic and placebo groups was the confounding effect of NSAIDs, which could mask any improvement attributed to probiotics. The highest pain score improvement was observed in the probiotic subgroup group that did not use NSAIDs (Table 4). However, as the sample size was small, the reduction was not significantly different from that in the placebo group. In addition, as evidenced by the better scores among NSAID users especially in the placebo group as compared with the probiotic group, the effect of probiotics, if any, was less significant than that of NSAIDs. A study comparing the effect of probiotics alone versus NSAIDs would provide a clearer

Table 4. Changes in quality of life scores after stratification by NSAID use.

Quality of life	*Mean* (*SD*)	Did not take NSAIDs			Took NSAIDs		
		Probiotic ($n=15$)	Placebo ($n=12$)	p^a	Probiotic ($n=19$)	Placebo ($n=22$)	p^a
Pain score (VAS)	Changes	−2.8 (2.6)	−2.6 (2.8)	0.84	−2.5 (2.4)	−2.4 (3.3)	0.95
Severity score (VRS)	Changes	−0.3 (0.52)	−0.5 (0.58)	0.58	−0.4 (0.8)	−1.0 (1.0)	**0.03**
Physical health score	Changes	4.0 (18.6)	11.3 (20.2)	0.34	4.8 (25.6)	4.4 (21.6)	0.96
Mental health score	Changes	8.4 (23.6)	9.9 (21.9)	0.87	7.2 (15.2)	4.0 (17.9)	0.55

SD: standard deviation; NSAID: nonsteroidal anti-inflammatory drug; VAS: visual analog scale; VRS: verbal rating scale.
[a]Using nonparametric independent samples Mann–Whitney U test.

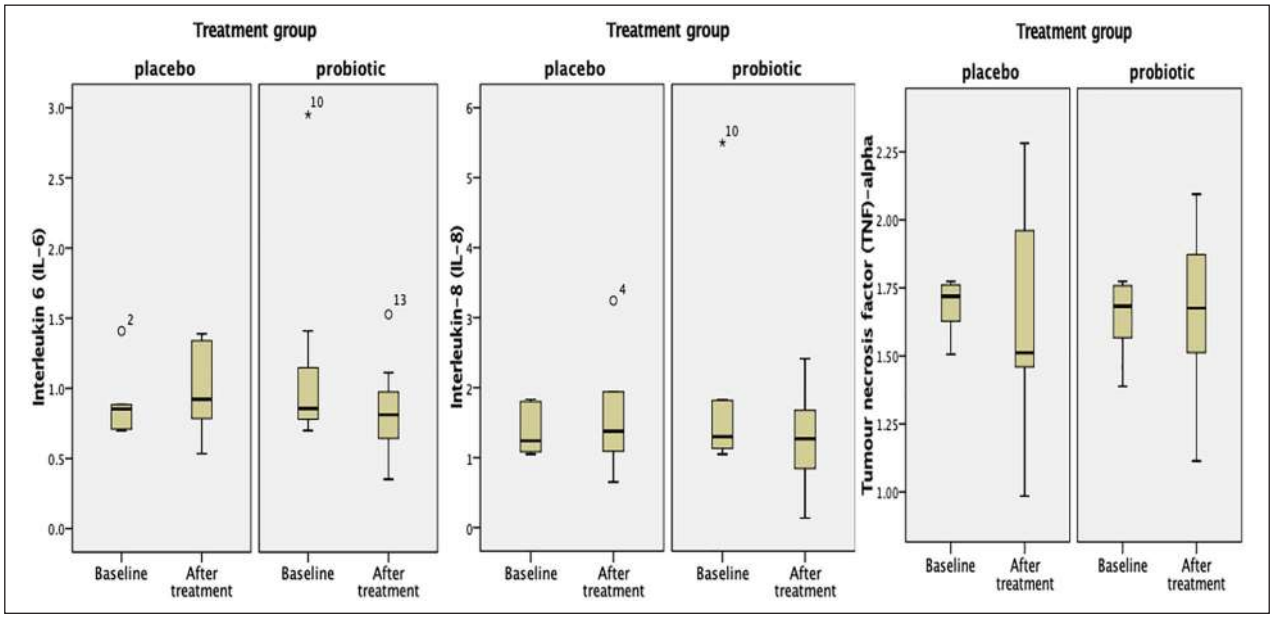

Figure 2. Boxplot showing concentration of inflammatory markers (IL-6, IL-8, and TNF-α) at baseline and after treatment comparing between treatment groups (placebo, $n=6$; probiotic, $n=8$).
$p > 0.05$, using independent Mann–Whitney U test.

picture, but this is not ethically possible at present because NSAIDs are the current standard treatment for dysmenorrhea and the pain can be severe; therefore, inhibiting access to NSAIDs is deemed unethical. Regarding inflammatory cytokines, it is possible that the differences in concentration between baseline and post-treatment levels were minute, especially when the samples were derived from the serum rather than the local endometrial or vaginal fluid. Ideally, a study using a highly sensitive immunoassay and larger sample size can detect minute changes; however, this study could not achieve this owing to budget constraints.

This double-blind randomized-controlled trial compared the effects of oral probiotics and placebo in women with primary dysmenorrhea. The double-blind design reduced observer bias, and the randomization process ensured similarity in baseline characteristics between the groups. Three months of supplementation was thought to be sufficient to produce an effect over three menstrual cycles, as this duration is usually prescribed for oral contraceptive pills or NSAIDs. The broad inclusion criteria enhanced external validity, and low exclusion ($n=6/78$) and dropout rates ($n=11/72$) ensured internal validity. The use of an intention-to-treat analysis further minimized the risk of bias and retained the randomization effect. Nevertheless, the impact on the intestinal microbiota was not tested, which is one of the limitations of this trial. In healthy patients, oral probiotics did not significantly affect fecal microbiota diversity.[23] Other studies have shown that oral probiotic supplementation during pregnancy promoted a healthy vaginal microbiota that suppressed preterm labor.[24] Probiotics have also been shown to positively modulate female microbiota and alleviate endocrine and fertility-related disorders.[25]

To our knowledge, this is the first study to assess the role of probiotics in primary dysmenorrhea. Previous trials have demonstrated the beneficial effect of oral probiotics in reducing dysmenorrhea and the severity of symptoms in

patients with endometriosis. In a randomized-controlled trial that investigated the effect of *Lactobacillus gasseri GG* on endometriosis symptoms, menstrual pain was significantly reduced in the *Lactobacillus* group compared with the placebo.[17] Another pilot randomized-controlled trial of 37 women with endometriosis also reported significant improvement in pain score after use of oral *Lactobacillus* LactoFem for 8 weeks compared with placebo.[18] However, the effects of probiotics have not been tested in women with primary dysmenorrhea. Many previous trials on primary dysmenorrhea have used alternative therapies, such as herbal remedies, behavioral interventions, exercise, and acupuncture, which showed improvement in menstrual pain; however, many of the studies had unclear protocols or low methodological quality; hence, their results were inconclusive.[26–28] Their assessments were also largely based on pain scores, and many did not assess the quality of life using specific questionnaires, such as those used in this study.[29–31] Other studies that assessed the quality of life of patients with primary dysmenorrhea were limited to observational studies and used different questionnaires.[1–3] A questionnaire that is sensitive enough to assess conditions like primary dysmenorrhea is yet to be decided.

Previous studies have identified the possible mechanisms by which oral probiotics may improve inflammatory conditions. van Baarlen et al.[16] showed that the colonization of the gastrointestinal mucosa by probiotic bacteria altered the expression of many genes involved in mucosal immunity and correlated significantly with the processes involved in regulating immune responses. In another study, *Lactobacillus rhamnosus* was reported to reduce proinflammatory TNF-α production in gram-positive bacteria, as evidenced by reduced level of C-reactive protein.[32] In women with polycystic ovarian syndrome, oral probiotic supplementation has been shown to reduce the levels of inflammatory markers such as IL-6, IL-10, C-reactive protein, and TNF-α.[33] A similar positive effect was also seen in patients with major depressive disorder, where oral probiotic supplementation led to a reduction in the urinary cortisol levels and significant improvement in depressive symptoms.[34] Other studies have shown that probiotics can affect metabolism and modulate inflammatory responses in female reproductive processes. A systematic review of the effects of probiotics in pregnancy showed a positive effect of probiotics on maternal metabolism, such as a reduction in C-reactive protein, fasting glucose, and the rate of preeclampsia.[35] Another large prospective cohort study also found a reduction in the rates of preterm delivery and preeclampsia in women who were administered probiotic milk during late pregnancy.[36]

Therefore, probiotic supplementation has a positive effect on inflammatory conditions.

There is growing evidence on the association between gut dysbiosis, increased estrogen reabsorption, and dysmenorrhea. Gut dysbiosis refers to any perturbation in the diversity and/or number of healthy populations of intestinal microbiota that can occur through diet, age, ethnicity, medication, smoking, and alcohol intake.[37] Gut dysbiosis affects beta-glucuronidase enzyme activity, which is the main deconjugation pathway of estrogen in the intestinal microbiota. This, in turn, affects estrogen metabolism and exacerbates estrogen-driven conditions like endometriosis, which is another major cause of dysmenorrhea.[38] The intestinal microbiota regulate estrogen metabolism via the estrogen–gut axis and estrobolome, and dysfunctions in these mechanisms can cause gynecological conditions such as endometriosis, infertility, chronic pelvic pain, and dysmenorrhea.[37,39]

In addition, endometriosis has been associated with a deficiency in *Lactobacillus* and proliferation of gram-negative organisms in many animal and human studies.[39–41] Given the potential of probiotic supplementation to reverse gut dysbiosis and restore estrogen homeostasis, oral probiotics can be used to treat gynecological conditions, especially disorders that have an inflammatory origin and are estrogen-driven, like primary dysmenorrhea.

However, this study had some limitations. The sample size was small; hence, it was underpowered to detect a significant difference in outcomes such as inflammatory markers and the frequency of NSAID use. The confounding effects of NSAID use were not considered. Second, although the compliance rate was reported to be >90% in each group, this was done via self-reporting, which is prone to reporter bias. We also did not measure the evolution of quality-of-life parameters per month and measured them only at two time points, before and after the 3-month treatment. Any benefit observed in the first 2 months could have been missed or lost at the end of treatment as a result of a lack of compliance. Furthermore, as pain in primary dysmenorrhea typically occurs more than 24 to 72 h, the SF12v2 questionnaire, which inquired about one's experience over a monthly period, might not be able to capture the impact of pain during these particular days.

Given the increasing evidence on the benefits of probiotics in reproductive and general health conditions, our study aimed to assess the effect of probiotics on primary dysmenorrhea, a common ailment affecting women of reproductive age that constitutes a major health and economic burden owing to its impact on workplace/school productivity. A previous metabolomic study of primary dysmenorrhea revealed a reduction in progesterone levels and several metabolomic conditions that could explain the mechanism of increased PG expression and pain perception.[42] Current strategies rely on the use of analgesics, mainly NSAIDs, which offer symptomatic relief but do not target the underlying pathogenesis. Probiotics rich in *Lactobacillus* promote a healthy gut environment and have been proven to be effective against inflammatory diseases such as eczema, irritable bowel syndrome, and endometriosis.[15,25,43] These benefits of

probiotics may be extrapolated to primary dysmenorrhea since this condition is also inflammatory in origin, although its exact pathogenesis is still unclear.

Conclusion

In conclusion, the tested oral probiotics did not affect the quality of life or levels of inflammatory cytokines in women with primary dysmenorrhea. However, the probiotics showed the potential to reduce NSAID use and improve pain and mental health; however, these results need to be further elucidated in larger trials, preferably using non-NSAID analgesics such as paracetamol or tramadol.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, approved by the Institutional Ethics Committee of the National University of Malaysia (FF-2018-204, 5 June 2018), and registered at ClinicalTrials.gov (NCT04119011). Written informed consent was obtained from all participants involved in the study.

Consent for publication

All subjects involved in the study consented for publication of their data.

Author contribution(s)

Izyan Atiqah Zakaria: Data curation; Formal analysis; Project administration; Visualization; Writing—original draft; Writing—review & editing.
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Availability of data and materials

The data sets used in this study can be found in the supplementary files submitted along with the article. Please contact the corresponding authors for further details.

Disclosure

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Supplemental material

Supplemental material for this article is available online.

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