Effect of Oral Lactoferrin in The Treatment of Bacterial Vaginosis

Salma Ashraf Nassar[1], Mohamed Elmandouh Mohamed[2], Mohamed Hesham Saeed Abdelsalam[3], Rasha Medhat Abdel Hady[4]
[1] Lecturer of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Egypt.
[2]Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Egypt.
[3]M.B.B. Ch, Faculty of Medicine, Alexandria University, Egypt.
[4]Assistant Professor of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Egypt.

Corresponding author:
Salma Ashraf Nassar
Phone No.: +201001673661
E-mail: Salma_Nassar@ymail.com

Abstract

Background : Bacterial vaginosis (BV) is the most common cause of vaginal discomfort in women. It is characterised by abnormal vaginal microbiota with a depletion of lactobacilli and predominance of anaerobic microorganisms, mainly Gardnerella vaginalis and Atopobium vaginae. Although antibiotics represent an effective therapeutic option in the short-term, recurrent infections still remain a serious problem.

Objectives: This study aimed to characterize the bacterial biota in women affected by bacterial vaginosis (BV) and evaluate the effect of combined orally administered lactoferrin and antibiotic treatment (Metronidazole) versus placebo and antibiotic treatment (Metronidazole) on the vaginal bacterial biota.

Methods: This double-blind randomized controlled clinical trial was conducted at the Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University Maternity Hospitals from December 2021 to December 2023 and included Sixty women diagnosed with BV and randomly divided into two groups: Group A (study group) who received 100 mg of oral lactoferrin plus Metronidazole, and Group B (control group) who received a placebo plus Metronidazole. After excluding seven participants for various reasons, 27 women in Group A and 26 in Group B completed the study.

Results: Both treatment methods were effective for bacterial vaginosis (BV). However, the lactoferrin group showed a significantly greater improvement in clinical signs and symptoms compared to the placebo group after 10 days of treatment. Only 2 participants (7.4%) in the lactoferrin group had vaginal discharge, whereas 8 participants (30.8%) in the placebo group did ($p<0.05$). The cure rate was 85.2% in the lactoferrin group compared to 69.2% in the placebo group, and the recurrence rate was 14.8% in the lactoferrin group versus 30.8% in the placebo group. There were no significant differences between the groups in terms of age, BMI, contraceptive method, history of recurrent BV, sexual activity frequency, baseline clinical signs, symptoms, and vaginal discharge samples.

Conclusions: In conclusion, this study evaluated the effectiveness of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care. The intervention significantly improved BV symptoms, including vaginal discharge, clue cell presence, and fishy odour. However, the differences between the intervention and standard care groups were statistically insignificant. We are aware that no firm conclusions can be drawn about the efficacy of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care from the results derived from such a small-sized study.

Key words: Bacterial Vaginosis, Oral Lactoferrin.

Introduction

Bacterial vaginosis (BV) is a prevalent gynecological condition characterized by an imbalance in the vaginal microbiota, affecting approximately 40-50% of women worldwide (1). The condition is marked by symptoms such as vaginal discharge, odor, and discomfort, significantly impacting the quality of life.(2) The pathogenesis of BV involves a reduction in the population of Lactobacillus species, which are crucial for maintaining a healthy vaginal environment through mechanisms such as lactic acid production, enhancement of the host's innate immune system, and the production of antimicrobial compounds like hydrogen peroxide and bacteriocins (3)

Despite the widespread use of antibiotics, such as Metronidazole, in the treatment of BV, recurrence rates remain alarmingly high (4). Approximately 25% of women experience a second episode of BV within four weeks of treatment, and long-term recurrence rates can exceed 70% (5). The reliance on antibiotics also brings potential downsides, including the development of antibiotic resistance and disruption of the natural vaginal flora. These challenges highlight the urgent need for alternative therapeutic strategies that are both effective and have fewer adverse effects (6).

Lactoferrin, an iron-binding glycoprotein belonging to the transferrin family, has emerged as a promising candidate in this context (7). Produced and stored in secondary neutrophil granules, lactoferrin is released during neutrophil activation and degranulation. It exhibits bacteriostatic and bactericidal properties by binding to iron, thereby limiting its availability to bacteria and inhibiting their growth. Moreover, lactoferrin has immunomodulatory effects and can disrupt bacterial cytoplasmic membranes, enhancing its antimicrobial efficacy. The synergistic effects of lactoferrin when combined with immunoglobulin A, lysozyme, antibiotics, and other drugs further support its potential utility in treating infections (5, 8).

Given these properties, lactoferrin's role in managing BV warrants thorough investigation. This study aims to evaluate the effectiveness of oral lactoferrin combined with Metronidazole in treating BV compared to the standard antibiotic treatment alone. By characterizing the bacterial biota in women affected by BV and assessing clinical outcomes, this research seeks to determine whether lactoferrin can enhance treatment efficacy and reduce recurrence rates.

Patient and method

After ethical committee approval and written consents from the patients, this double-blind randomized controlled clinical trial was performed on a total of Sixty women diagnosed with BV attending the outpatient clinic at Ain Shams University Maternity Hospital in period between December 2021 to December 2023.

Study Population: All included women were randomly (1:1 ratio) allocated to one of the two experimental groups:

- **Group A (study group):** to whom lacto-

ferrin 100 mg (Pravotin® sachets 100mg, Hygint, Egypt) 2 sachets/day for 5 days followed by 1 sachet/day for 10 consecutive days plus antibiotic treatment "metronidazole" 500mg twice daily for 7 days.

- **Group B (control group):**to whom placebo 2 sachets/day for 5 days followed by 1 sachet/day for 10 consecutive days plus antibiotic treatment "metronidazole" 500mg twice daily for 7 days.

Inclusion criteria included sexually active women aged 18-45 years, with a BMI of 18-25 kg/m², regular menstrual cycles, and symptomatic acute BV diagnosed according to Amsel's criteria and Nugent's standardized method of Gram-stain interpretation.

Exclusion criteria included women with medical conditions as diseases like diabetes mellitus led to decrease the immunity and increase the risk of bacterial infection, women with active infections from other pathogens due to change in the vaginal PH and mixed infection can't test the effect of lactoferrin on two modifiable risk factors, pregnant or breastfeeding due to hormonal fluctuations that lead to change in the vaginal environment, vaginal PH and increase the risk of vaginal infection, women who had recent antibiotic or hormone treatments as they lead to decreased immunity and change in the vaginal normal bacterial flora, women with Nugent score <7, women with gynecological conditions causing bleeding as they interfere with the result of vaginal smear and change the vaginal PH and women with allergies to study medications, or refused participation.

Diagnostic criteria of BV:

Amsel's criteria: Amsel et al. (9) described four criteria for the diagnosis of BV in clinical settings. Any combination of three of its four diagnostic criteria (abundant creamy, grey-white adherent vaginal discharge, rotten fish odor - spontaneous or alkali induced -, pH > 4.5, microscopic detected presence of clue cells (exfoliated vaginal epithelial cells covered by G. vaginalis) leads to the diagnosis of BV (9).

In addition to Amsel criteria, BV was also diagnosed by Nugent's standardized method of Gram-stain interpretation in which the Nugent method consisted of microscopic evaluation of bacterial morphotypes to score the overall character of the vaginal flora. Nugent scores range from 0-10, based on the prevalence of 3 bacterial morphotypes that roughly correspond to Lactobacillus, G vaginalis or Bacteroides, and Mobiluncus. The different cell types are counted (Mobiluncus spp., G. vaginalis/Bacteroides spp. And Lactobacillus spp.) and a score between 0 and 10 is obtained; whereby a score of 7–10 corresponds to BV, a score of 4–6 is considered intermediate (partial BV) and a score of 0–3 indicates an undisturbed vaginal microflora. Intermediate scores may indicate the development of BV or a woman that is being cleared of this disease entity; however, these ''intermediate flora'' remains contentious (10).

However, Amsel criteria and Nugent scoring are considered equally efficacious in diagnosing bacterial vaginosis (11).

Sample size Justification:

Group sample sizes of 26 cases per group totaling 52 cases achieve 80% power to reject the null hypothesis of zero effect size when the population effect size is 0.80 and the significance level (alpha) is 0.05 using a two-sided two-sample equal-variance t-test. A 10% excess of cases should be included to compensate for the dropouts, making the total sample size 60 cases divided equally between the two groups

Randomization was done using computer generated randomization sheet.

Allocation and concealment: were done by use of sealed opaque envelopes that were given to a third party (nurse) who assigned the women to study arms. Each woman was invited to pull out an envelope. According to the number inside her envelope, women were allocated to either group 1 or group 2 according to a computer- generated random list.

Detection bias was avoided by blinding patients and assessor to the groups. The sachets in both groups were similar in shape and package and administered anonymously with coding by a midwife colleague with no knowledge of the codes. Final assessment was performed by another colleague who had no information about the groups.

Ethical Considerations: The patient data were anonymous. Data presentation was not be by the patient's name but by diagnosis and patient confidentiality was protected. An informed consent was taken from all participants, it was in Arabic language and confirmed by date and time. confidentiality was preserved by assigning a number to patients initials and only the investigator knew it. Protocol approval by the council of the OB/GYN department at Ain Shams University was done.

Study Procedures:

After a written consent was taken, the recruited women were subjected to a thorough clinical examination that included a full history as well as general, abdominal, and pelvic exams.

History taking: including (personal history, age, obstetric history, medical history, surgical history, past history and family history).

Clinical examinations: include general, abdominal, pelvic exams and assessment of clinical signs and symptoms of vaginosis, and vaginal discharge sampling were performed at each intervention point (base line and after 10 days of treatment).

Investigations: including (labs; CBC, blood group, liver and kidney functions, coagulation profile & imaging; Transvaginal ultrasound).

Sample collection:

Vaginal discharge samples were obtained from the lateral vaginal wall and the posterior vaginal fornix using sterile cotton-tipped swabs. For each participant, two vaginal smears were collected at baseline and after 10 days of treatment.

Vaginal smears were used to assess the BV status: microscopic examination of the fresh smear (detection of clue cells and Gram staining), whiff amine test. Vaginal fluid was measured during each visit using pH test strips.

According to Amsel's criteria, the whiff-amine test was carried out. In detail, the presence of a 'fish odor', attributable to the production of volatile amines, were evaluated by adding a drop of 10% KOH directly to the glass surface, and the presence of fish odor was detected by the researcher's sense of smell. The Amsel's criteria were also performed at the base line and after 10 days of treatment sampling times (9).

According to Nugent scoring system, Lactobacilli are given a score of 0 if found in normal amounts and a numeric score if low in quantity or absent. The other organisms are scored based on numbers present. The sum of the three subscores is the patient's Nugent score: 0-3 is normal, 4-6 is intermediate, and 7-10 indicates bacterial vaginosis (10).

Outcome measures:

- **Primary outcome:** Complete cure rate.
- **Secondary outcomes:** Non recurrence of infection within 4 weeks

Statistical analysis: Analysis is to be performed using SPSS for windows v20.0, Data to be presented in terms of range, mean and standard deviation (for numeric parametric variables); range, median and inter-quartile range (for numeric non-parametric variables); or number and percentage (for categorical variables). Difference between two independent groups is to be analyzed using independent student's t-test as well as the mean difference and its 95% CI (for numeric parametric variables); or chi-squared test as well as the risk ratio and its 95% CI (for categorical variables). Binary logistic regression analysis is to be performed for estimating the association between good/poor response and

the measured variables ROC curves are to be constructed for estimating the validity of measured variables as predictors of good or poor response validity is to be presented in terms of sensitivity, specificity, positive and negative predictive values and their corresponding 95% Cis significance level is set at 0.05.

RESULTS

During this study, 85 patients were assessed for eligibility and 60 patients were included in the study (30 in each group). Of all eligible patients, 16 patients were excluded from the study based on the inclusion criteria and 9 patients refused to participate in of the study. However, three patients dropped out in lactoferrin group and 4 patients dropped in the placebo group during follow-up. Ultimately, the analysis was based on the data of 27 women in lactoferrin group and 26 women in the placebo group.

Table (1): Comparison between study group and control group according to clinical signs and symptoms of vaginosis at baseline and after 10 days of treatment.

Baseline clinical signs and symptoms of vaginosis	Study group (n=27)	Control group (n=26)	Test value	P-value
Vaginal discharge	27 (100.0%)	26 (100.0%)	0.000	1.000
Signs and symptoms of vaginitis after 10 days of treatment				
No signs and symptoms	23 (85.2%)	18 (69.2%)	1.122	0.289
Vaginal discharge	4 (14.8%)	8 (30.8%)		

(t) Student t-test; (p) probability value.

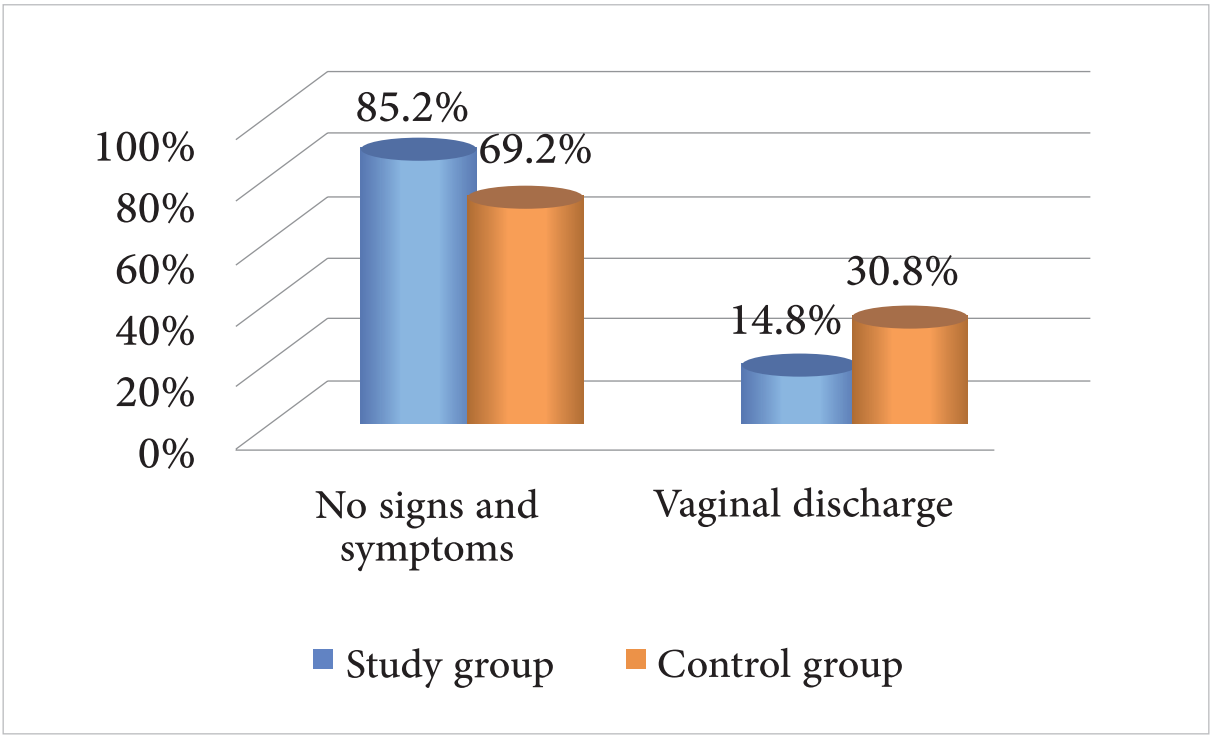

Figure (1): Comparison between Study group and Control group according to Signs and symptoms of vaginitis after 10 days of treatment

Table (1) and Figure (1) show that all studied patients presented with vaginal discharge. However, signs and symptoms after 10 days of treatment were improved compared to baseline in both groups.

Table (2): Comparison between baseline and after 10 days of treatment according to clinical signs and symptoms of vaginosis in the study group.

Clinical Signs And Symptoms of Vaginosis	Study group (n= 27)		t	p
	Baseline	After 10 days of treatment		
No signs and symptoms	0 (0.0%)	23 (85.2%)	13.587	**<0.001***
Vaginal discharge	27 (100.0%)	4 (14.8%)		

(t) Student t-test; (p) probability value; (*) statistically significant result.

Table (2) shows that there was statistically significant higher frequency of improved signs and symptoms after 10 days of treatment in the study group, compared to baseline.

Table (3): Comparison between baseline and after 10 days of treatment according to clinical signs and symptoms of vaginosis in the control group.

Clinical Signs And Symptoms of Vaginosis	Control group (n= 26)		t	p
	Baseline	After 10 days of treatment		
No signs and symptoms	0 (0.0%)	18 (69.2%)	32.8	**<0.001***
Vaginal discharge	26 (100%)	8 (30.8%)		

(t) Student t-test; (p) probability value; (*) statistically significant result.

Table (3) shows that there was statistically significant higher frequency of improved signs and symptoms after 10 days of treatment in the control group, compared to baseline.

Table (4): Comparison between baseline and after 10d of treatment according to vaginal discharge sampling in the study group.

Vaginal discharge sampling	Study group (n=27)		Test value	p-value
	Baseline	After 10d of treatment		
pH Baseline				
pH 4	0 (0.0%)	2 (7.4%)	15.373	0.002*
pH 5	5 (18.5%)	17 (63.0%)		
pH 6	21 (77.8%)	8 (29.6%)		
pH 7	1 (3.7%)	0 (0.0%)		
pH 8	0 (0.0%)	0 (0.0%)		
Clue cells Baseline				
No clue cells	14 (51.9%)	24 (88.9%)	7.194	0.007*
Positive clue cells	13 (48.1%)	3 (11.1%)		
Fishy Odour Baseline				
Fishy Odour	19 (70.4%)	1 (3.7%)	22.95	<0.001**
No Fishy Odour	8 (29.6%)	26 (96.3%)		

Using: McNemar's test *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table (4) shows that there was statistically significant reduction mean of pH the after treatment than baseline. Also, there was high frequency of improved fishy odor after treatment compared to baseline with significant reduction in the positive clue cells after treatment comparing to baseline.

Table (5): Comparison between baseline and after 10d of treatment according to vaginal discharge sampling in the control group.

Vaginal discharge sampling	Control group (n=26)		Test value	p-value
	Baseline	After 10d of treatment		
pH Baseline				
pH 4	0 (0.0%)	1 (3.8%)	15.021	0.005*
pH 5	3 (11.5%)	14 (53.8%)		
pH 6	21 (80.8%)	10 (38.5%)		
pH 7	2 (7.7%)	0 (0.0%)		
pH 8	0 (0.0%)	1 (3.8%)		
Clue cells Baseline				
No clue cells	13 (50.0%)	23 (88.5%)	7.312	0.007*
Positive clue cells	13 (50.0%)	3 (11.5%)		
Fishy Odour Baseline				
Fishy Odour	19 (73.1%)	5 (19.2%)	13.077	0.002*
No Fishy Odour	7 (26.9%)	21 (80.8%)		

Using: McNemar's test *p-value <0.05 is significant; **p-value <0.001 is highly significant.

Table (5) shows that there was statistically significant reduction mean of pH the after treatment than compared to the baseline. Also, there was high frequency of improved fishy odour after treatment comparing to baseline with significant reduction in the positive clue cells after treatment comparing to baseline.

Table (6): Comparison between study group and control group according to outcome.

Outcome	Study group (n=27)	Control group (n=26)	Test value	P-value	Sig
Cured	23 (85.2%)	18 (69.2%)	1.925	0.165	NS
Resistant	**4 (14.8%)**	**8 (30.8%)**			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate.

Table (6) shows that the higher frequency of cured cases was 23 patients (85.2%) in the study group, compared to 18 patients (69.2%) in control group, with p-value (p>0.05).

Table (7): Comparison between study group and control group according to recurrence of infection within 4 weeks.

Recurrence of infection within 4 weeks	Study group (n=23)	Control group (n=18)	Test value	P-value	Sig
No Recurrence of infection	19 (82.6%)	14 (77.8%)	2.073	0.355	NS
Recurrence after 4 weeks	4 (17.4%)	4 (22.2%)			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate.

Table (7) shows that the recurrence of infection after 4 weeks was 4 patients (17.4%) in the study group, compared to 4 patients (22.2%) in the control group, but insignificant difference, with p-value (p>0.05).

DISCUSSION

Currently, the standard-of-care therapeutic approach for BV treatment is based on antibiotic administration, often metronidazole and/or clindamycin (12). Although the antibiotic treatment is effective in about 80% of BV cases, a high recurrence rate (>50%) is reported within 6–12 months. In addition, prolonged and repeated antibiotic treatments facilitate the development of resistant pathogens. In this context, resistance to both metronidazole and clindamycin is well documented among G. vaginalis isolates (13, 14, 15), therefore complementary and/or alternative therapeutic approaches are needed, such as oral lactoferrin. a glycoprotein with antimicrobial and immunomodulatory properties, are being explored.

Since bacterial vaginosis represents major conflict and may be associated with a significant number of obstetric and gynecologic complications, such as preterm labor and delivery, preterm premature rupture of membranes, spontaneous abortion, chorioamnionitis, endometritis, post-Caesarean delivery wound infections, postsurgical infections, and subclinical pelvic inflammatory disease (16), evaluating the efficacy of a probiotic Lactobacillus mixture in combination with bovine lactoferrin as adjuvant to drug therapy with oral metronidazole in adult women with BV was highlighted as a main point of interest (11).

Consequently, this study was conducted and aimed to characterize the bacterial biota in women affected by bacterial vaginosis (BV) and evaluate the effect of combined orally administered lactoferrin and antibiotic treatment (Metronidazole) versus placebo and antibiotic treatment (Metronidazole) on the vaginal bacterial biota.

This double-blind randomized controlled clinical trial was conducted at at Ain Shams University Maternity Hospital (ASUMH) from December 2021 to December 2023 and performed on a total of Sixty women diagnosed with bacterial vaginosis.

The current study revealed that there were no significant demographic differences between the groups, including age, BMI, contraceptive use, infectious disease history and sexual activity among the study groups. This ensured comparability for assessing the intervention's impact on BV.

Regarding the clinical signs and symptoms of bacterial vaginosis, our study results revealed that all patients in both groups presented with vaginal discharge. However, after 10 days of treatment, the clinical signs and symptoms in study group and control group significantly improved and decreased to 14.8% and 30.8%, respectively ***(p value <0.001)***, compared to the baseline with no significant difference between the two group after 10 days of treatment (p value = 0.289).

Regarding the vaginal discharge sampling, our study results revealed that there were no statistically significant differences between study group and control group according to baseline vaginal discharge sampling regarding pH Baseline, Clue cells Baseline and Fishy Odour Baseline, with p-value ($p>0.05$). However, 10 days after treatment, the vaginal discharge sampling revealed significant improvement and reduction of the PH, Clue cells Baseline and Fishy Odour Baseline in both groups with no insignificant difference between groups, with p-value ($p>0.05$).

Regarding the outcome, our study results revealed that 23 patients (85.2%) in the study group were cured, compared to 18 patients (69.2%) in control group, and the recurrence of infection after 4 weeks was 4 patients (17.4%) in the study group, compared to 4 patients (22.2%) in the control group. however, the differences were statistically insignificant, with p-value ($p>0.05$).

It is important to note that vaginal Lactobacillus species can form a protective barrier against pathogen invasion. The metabolites they secrete into the cervicovaginal fluid play a crucial role in inhibiting bacterial and viral infections (17). Additionally, the low vaginal pH (< 4.5) resulting from lactic acid production by Lactobacillus species helps to suppress the growth of pathogenic microorganisms that cause vaginal dysbiosis. The vaginal pH is a key factor in the high incidence of BV among women of reproductive age. Various adjuvant treatments, including ascorbic acid, Lactobacillus strains, and probiotics, have been explored to lower vaginal pH and reduce the recurrence of BV (18, 5).

However, treatment remains unsatisfactory until the pathogenesis of BV is fully understood. Clinicians use different regimens to treat BV, typically involving antibiotics like metronidazole or clindamycin, administered orally or topically. Although many women initially respond to these antibiotics, BV recurs in 11–29% of cases within a month, and an adherent G. vaginalis biofilm often remains post-therapy. Bacteria within biofilms exhibit higher resistance to antibiotics compared to their free-floating counterparts (19).

A promising therapeutic approach involving lactoferrin administration for treating BV has been proposed. Pino et al. (5) conducted an open prospective randomized trial to investigate the bacterial biota of women with BV and evaluate the effects of two different lactoferrin concentrations (100 mg and 200 mg vaginal pessaries) on the composition and dynamics of the vaginal microbiota. The study found that vaginal lactoferrin administration altered the vaginal microbiota in BV patients. Both 100 mg and 200 mg lactoferrin pessaries significantly reduced the presence of bacteria associated with BV, such as Gardnerella, Prevotella, and Lachnospira, while increasing Lactobacillus species. The balance of the bacterial biota was maintained up to 2 weeks after treatment only in women who received the 200 mg lactoferrin pessaries. However, this study is limited by the absence of control group and also, using vaginal route may be effective, compared to the orally administered lactoferrin in our study.

The primary mechanism of lactoferrin's action appears to be its high-affinity iron sequestration (Kd 10–20), creating a bacteriostatic, iron-depleted environment (17). In a later study, Pino et al. (20) evaluated the susceptibility of 71 presumptive Gardnerella vaginalis clinical isolates to metronidazole and clindamycin, and investigated the in vitro antimicrobial activity of Metrodora Therapeutics bovine Lactoferrin (MTbLF) at various concentrations. The study found that lactoferrin's antimicrobial effect was dose-dependent rather than strain-dependent, indicating that the tested strains could use iron for growth. G. vaginalis strains can utilize several iron-containing compounds, including iron salts, hemin, hemoglobin, and human lactoferrin, but not bovine lactoferrin. The inability of bovine lactoferrin to support G. vaginalis growth was confirmed with multiple presumptive clinical isolates.

The specificity of G. vaginalis lactoferrin binding proteins for human lactoferrin suggests that bovine lactoferrin is preferable to recombinant human lactoferrin for BV therapeutics. Additionally, a synergistic effect was observed when MTbLF was used in combination with clindamycin, suggesting that MTbLF could enhance the effectiveness of traditional antimicrobials and help combat antimicrobial resistance.

Furthermore, Russo et al. (11) conducted a double-blind, randomized clinical trial that assessed the efficacy of a probiotic mixture, including Lactobacillus acidophilus GLA-14 and Lactobacillus rhamnosus HN001, combined with bovine lactoferrin, as an adjunct therapy to metronidazole in women with recurrent BV. The study revealed significant improvements in symptoms (vaginal discharge and itching), Nugent scores, and recurrence rates with the probiotic mixture and lactoferrin combination. This alternative approach may represent a safe and effective remedy for restoring healthy vaginal microbiota and preventing recurrent BV.

Lactoferrin may be an effective alternative remedy for preventing BV as it helps restore a healthy vaginal microbiota, predominantly composed of lactobacilli (11). Additionally, Bertuccini et al. (21) demonstrated that bovine lactoferrin, when combined with L. acidophilus GLA-14 and L. rhamnosus HN001, both alone and together, could enhance the biofilm formed by these strains on both abiotic surfaces and HeLa cell monolayers. This promising result supports the potential of a symbiotic mixture of lactoferrin and lactobacilli for promoting vaginal health.

A previous clinical study by De Alberti et al. (22) found that oral administration of Respecta significantly increased vaginal colonization by L. acidophilus GLA-14 and L. rhamnosus HN001 in healthy women after two weeks of treatment. Conversely, the placebo did not result in any increase in lactobacilli levels in the vagina of the treated women. Notably, the levels of both strains continued to rise one week after the cessation of oral intake, suggesting a prolonged persistence of lactobacilli in the vagina.

Extensive research supports the pathogenic role of G. vaginalis and suggests that vaginal Gardnerella spp. biofilm can lead to the ineffectiveness of conventional antibiotic treatments and high recurrence rates, implying that other bacteria within the biofilm may act as opportunistic secondary invaders. Furthermore, bacteria in biofilms exhibit different responses to antibiotics compared to their free-floating (planktonic) forms, with antibiotic resistance believed to be a contributing factor to persistent and recurrent BV. Saunders et al. (23) found that in cases involving BV biofilms, strains of Lactobacillus might be able to disrupt and remove these biofilms, potentially reducing the reliance on antibiotics.

Moreover, lactobacilli supplementation significantly improved symptoms such as discharge and odor, as well as Nugent scores, during the intervention period (24). Anukam et al. (25) conducted a study where, after a 7-day treatment with metronidazole, participants were given either a probiotic oral formulation containing L. rhamnosus GR-1 and L. reuteri RC-14 or a placebo for 30 days. The study reported an initial cure rate of 88% for the probiotic group compared to 40% for the placebo group ($P<0.001$), with a significant increase in lactobacilli in vaginal swabs from the treated group. However, due to the limited amount of data, probiotics cannot yet be recommended for women with BV.

The benefits of using probiotics should be evaluated alongside known risk factors for BV, such as diet, douching, contraception type, and menstrual cycle. To sustain and maximize long-term benefits, BV should be managed as a chronic condition requiring both short-term and long-term treatments (19). The unclear causes of BV, high recurrence rates, sub-optimal treatment options, often inconsistent clinical advice, and distressing symptoms remain significant chal-

lenges in modern obstetrics and gynecology (26). Consequently, it is not surprising that women often resort to various self-help remedies to prevent further recurrences, and doctors are continually searching for new therapeutic strategies.

The differences between these studies and ours might be attributed to the use of a probiotic mixture, including Lactobacillus acidophilus GLA-14 and Lactobacillus rhamnosus HN001 combined with bovine lactoferrin (Respecta), the prolonged treatment period, and the different methodology. In the study by Russo et al. (11), Respecta or placebo was administered as one capsule per day for 10 consecutive days, repeated each month during a six-month follow-up period, starting on the first day of the menstrual cycle. This approach considers that menstrual blood increases vaginal pH and thereby heightens the risk of recurrences.

Although many healthcare providers might view vulvovaginal infections as relatively straightforward to treat, treatment failures and recurrent infections are common. Patients frequently receive repeated courses of the same ineffective treatments. However, for most women experiencing chronic or recurrent vaginal infections, there are evaluation and treatment approaches that can provide more satisfactory outcomes. In this context, the use of probiotics may be a viable therapeutic strategy (11).

These findings may underscore the potential of lactoferrin in managing BV, highlighting its role in reducing clinical signs and symptoms, particularly vaginal discharge. However, some variability in treatment response suggests the need for personalized approaches and further research to optimize treatment strategies.

The strength points of this study:

The strength points of this study are that it is double-blind randomized controlled clinical trial design, its setting at a single tertiary care center and presence compared control group. To the best of our knowledge, there is a paucity of studies in literature evaluating the bacterial vaginosis treatment with lactoferrin, compared with control group, and that represents a strength point of our study.

The limitations of the study

The findings of this study should be interpreted in light of its limitations firstly, including relatively smaller sample size relative to the previous studies, not being a multicentric study and this represents a significant risk of publication bias. Secondly, using only orally administrated route and small dose of the drug (lactoferrin 100 mg) which may underestimate the antimicrobial effect of Lactoferrin as the antimicrobial effect of Lactoferrin was dose-dependent and not strain-dependent (Pino et al., 2022). Thirdly, short period of treatment and follow up.

Conclusions

In conclusion, this study evaluated the effectiveness of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care. The intervention significantly improved BV symptoms, including vaginal discharge, clue cell presence, and fishy odour. However, the differences between the intervention and standard care groups were statistically insignificant.

We are aware that no firm conclusions can be drawn about the efficacy of oral lactoferrin supplementation combined with antibiotic treatment for treating bacterial vaginosis compared to standard care from the results derived from such a small-sized study. Nevertheless, the present study can burden the knowledge and shed some light on future prospective studies with larger sample sizes, high dose of lactoferrin mixed with probiotics and several lactobacilli strains with long period of treatment and follow up demonstrating the long-term effect of high dose of lactoferrin.

Conflict of interest

the candidate declared that there is no conflict of interest and the cost of the study was paid by the candidate.

REFERENCES

1. Mondal AS, Sharma R, Trivedi N. Bacterial vaginosis: A state of microbial dysbiosis. Medicine in Microecology. 2023;16:100082.
2. Bilardi JE, Walker S, Temple-Smith M, McNair R, Mooney-Somers J, Bellhouse C, et al. The burden of bacterial vaginosis: women's experience of the physical, emotional, sexual and social impact of living with recurrent bacterial vaginosis. PLoS One. 2013;8(9):e74378.
3. Khedkar R, Pajai S. Bacterial Vaginosis: A Comprehensive Narrative on the Etiology, Clinical Features, and Management Approach. Cureus. 2022;14(11):e31314.
4. Muzny CA, Sobel JD. Understanding and Preventing Recurring Bacterial Vaginosis: Important Considerations for Clinicians. Int J Womens Health. 2023;15:1317-25.
5. Pino A, Giunta G, Randazzo CL, Caruso S, Caggia C, Cianci A. Bacterial biota of women with bacterial vaginosis treated with lactoferrin: an open prospective randomized trial. Microb Ecol Health Dis. 2017; 28(1):1357417.
6. Gustin AT, Thurman AR, Chandra N, Schifanella L, Alcaide M, Fichorova R, et al. Recurrent bacterial vaginosis following metronidazole treatment is associated with microbiota richness at diagnosis. Am J Obstet Gynecol. 2022; 226(2):225. e1-.e15.
7. Ashraf MF, Zubair D, Bashir MN, Alagawany M, Ahmed S, Shah QA, et al. Nutraceutical and Health-Promoting Potential of Lactoferrin, an Iron-Binding Protein in Human and Animal: Current Knowledge. Biol Trace Elem Res. 2024; 202(1):56-72.
8. Wong SH, Francis N, Chahal H, Raza K, Salmon M, Scheel-Toellner D, Lord JM. Lactoferrin is a survival factor for neutrophils in rheumatoid synovial fluid. Rheumatology (Oxford). 2009; 48(1):39-44.
9. Amsel, Richard, Patricia A. Totten, Carol A. Spiegel, Kirk CS Chen, David Eschenbach, and King K. Holmes. "Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations." The American journal of medicine. 1983; 74 (1): 14-22.
10. Nugent, Robert P., Marijane A. Krohn, and Sharon L. Hillier. "Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation." Journal of clinical microbiology. 1991; 29(2): 297-301.
11. Russo, R., E. Karadja, and F. De Seta. "Evidence-based mixture containing Lactobacillus strains and lactoferrin to prevent recurrent bacterial vaginosis: a double blind, placebo controlled, randomised clinical trial." Beneficial microbes. 2019; 10(1): 19-26.
12. Verstraelen, Hans, and Alexander Swidsinski. "The biofilm in bacterial vaginosis: implications for epidemiology, diagnosis and treatment: 2018 update." Current opinion in infectious diseases. 2019; 32(1): 38-42.
13. de Souza, Daniele Maria Knupp, Cláudio Galuppo Diniz, Didier Silveira Castellano Filho, Laura Maria Andrade de Oliveira, Débora Martins Coelho, Luciana de Souza Talha, Thiago César do Nascimento, Alessandra Barbosa Ferreira-Machado, and Vânia Lúcia da Silva. "Antimicrobial susceptibility and vaginolysin in Gardnerella vaginalis from healthy and bacterial vaginosis diagnosed women." The Journal of Infection in Developing Countries. 2016; 10(9): 913-919.
14. Schuyler, Jessica A., Eli Mordechai, Martin E. Adelson, Jack D. Sobel, Scott E. Gygax, and David W. Hilbert. "Identifi-

cation of intrinsically metronidazole-resistant clades of Gardnerella vaginalis." Diagnostic microbiology and infectious disease. 2016; 84(1): 1-3.

15. Hopp, Thomas P., Klaudyna Spiewak, Maura-Ann H. Matthews, Zafeiria Athanasiou, Richard S. Blackmore, and Gary A. Gelbfish. "Characterization of proteolytic degradation products of vaginally administered bovine lactoferrin." Plos one. 2022; 17(5): e0268537.
16. Kulkarni, Priyanka, and Girija Wagh. "Prevalence of bacterial vaginosis in pregnant women and its association with adverse perinatal outcomes." Indian Journal of Obstetrics and Gynecology Research. 2020; 7(2): 179-184.
17. Lin, Ta-Chin, I-Ling Hsu, Wan-Hua Tsai, Yi-Chih Chu, Lung-Ching Kuan, Min-Syuan Huang, Wen-Ling Yeh, Ya-Hui Chen, Shan-Ju Hsu, and Wen-Wei Chang. "Improvement of bacterial vaginosis by oral lactobacillus supplement: A randomized, double-blinded trial." Applied Sciences. 2021; 11(3): 902.
18. Larsson, Per-Göran, Erik Brandsborg, Urban Forsum, Sonal Pendharkar, Kasper Krogh Andersen, Salmir Nasic, Lennart Hammarström, and Harold Marcotte. "Extended antimicrobial treatment of bacterial vaginosis combined with human lactobacilli to find the best treatment and minimize the risk of relapses." BMC infectious diseases. 2011; 11: 1-14.
19. Artym, Jolanta, and Michał Zimecki. "Antimicrobial and prebiotic activity of lactoferrin in the female reproductive tract: a comprehensive review." Biomedicines. 2021; 9(12): 1940.
20. Pino, Alessandra, Tommaso Mazza, Maura-Ann H. Matthews, Stefano Castellana, Cinzia Caggia, Cinzia L. Randazzo, and Gary A. Gelbfish. "Antimicrobial activity of bovine lactoferrin against Gardnerella species clinical isolates." Frontiers in Microbiology. 2022; 13: 1000822.
21. Bertuccini, Lucia, Rosario Russo, Francesca Iosi, and Fabiana Superti. "Lactobacilli and lactoferrin: Biotherapeutic effects for vaginal health." Journal of functional foods. 2018; 45: 86-94.
22. De Alberti, Davide, Rosario Russo, Fabio Terruzzi, Vincenzo Nobile, and Arthur C. Ouwehand. "Lactobacilli vaginal colonisation after oral consumption of Respecta® complex: a randomised controlled pilot study." Archives of gynecology and obstetrics. 2015; 292: 861-867.
23. Saunders, Sheri, Alan Bocking, John Challis, and Gregor Reid. "Effect of Lactobacillus challenge on Gardnerella vaginalis biofilms." Colloids and Surfaces B: Biointerfaces. 2007; 55(2): 138-142.
24. Laue, C., E. Papazova, A. Liesegang, A. Pannenbeckers, P. Arendarski, B. Linnerth, K. J. Domig, W. Kneifel, L. Petricevic, and J. Schrezenmeir. "Effect of a yoghurt drink containing Lactobacillus strains on bacterial vaginosis in women–a double-blind, randomised, controlled clinical pilot trial." Beneficial Microbes. 2018; 9(1): 35-50.
25. Anukam, Kingsley, Emmanuel Osazuwa, Ijeoma Ahonkhai, Michael Ngwu, Gibson Osemene, Andrew W. Bruce, and Gregor Reid. "Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo-controlled trial." Microbes and Infection. 2006; 8(6): 1450-1454.
26. Valenti, Piera, Luigi Rosa, Daniela Capobianco, Maria Stefania Lepanto, Elisa Schiavi, Antimo Cutone, Rosalba Paesano, and Paola Mastromarino. "Role of lactobacilli and lactoferrin in the mucosal cervicovaginal defense." Frontiers in immunology. 2018; 9: 338405.