Arch Gynecol Obstet
DOI 10.1007/s00404-017-4555-x

GENERAL GYNECOLOGY

The effect of prebiotic vaginal gel with adjuvant oral metronidazole tablets on treatment and recurrence of bacterial vaginosis: a triple-blind randomized controlled study

Sevil Hakimi[1] · Faranak Farhan[2] · Azizeh Farshbaf-Khalili[3] · Parvin Dehghan[4] · Yousef Javadzadeh[5] · Shamsi Abbasalizadeh[2] · Bagher Khalvati[6]

Received: 17 May 2017 / Accepted: 30 August 2017

Abstract
Purpose Bacterial vaginosis is a change in the normal vaginal bacterial flora that leads to loss of hydrogen peroxide producing lactobacilli and overgrowth of predominantly anaerobic bacteria. The present study was conducted to compare the effects of prebiotic vaginal gel with oral metronidazole tablet and metronidazole tablet alone on treatment and recurrence of bacterial vaginosis.
Methods The present triple-blind randomized clinical trial was conducted in Sadatmandi Hospital in Robat-Karim town, where 100 patients were randomly divided into intervention (receiving a 5 mg prebiotic vaginal gel applicator plus three 250 mg metronidazole tablets per day for 7 days) and control (receiving a 5 mg placebo vaginal gel applicator and three 250 mg metronidazole tablets per day for 7 days) groups. Then, patients were assessed for bacterial vaginosis on 90 ± 3 day after treatment. Data collected were analyzed in SPSS-21 using Chi square, repeated measures, and student's *t* tests at a significance level of $P < 0.05$.
Results The results obtained showed no significant difference between the two groups in terms of personal and social characteristics, clinical complaints, or laboratory markers. On the 10th day, healing rate based on Amsel and Nugent criteria was 76% in the intervention group and 30% in the control group [odds ratio (OR) 4.3; 95% confidence interval (CI) 2.7–9.4]. On the 90th day, healing rate was 84% in the intervention group and 62% in the control group (OR 3.7; 95% CI 1.3–8.9).
Conclusions Adjuvant treatment with prebiotic vaginal gel improves the efficacy of bacterial vaginosis treatment.

Keywords Prebiotic · Bacterial vaginosis · Amsel criteria · Nugent criteria · Recurrence

✉ Faranak Farhan
faranak.farhan39@yahoo.com

Sevil Hakimi
Hakimis@tbzmed.ac.ir

Azizeh Farshbaf-Khalili
Azizeh_farshbafkhalili@yahoo.com

Parvin Dehghan
Dehghan.nut@gmail.com

Yousef Javadzadeh
Javadzadehy@yahoo.com

Shamsi Abbasalizadeh
shamciabbasalizadeh@gmail.com

Bagher Khalvati
b_khalvati@yahoo.com

[1] Women's Reproductive Health Research Center, School of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Physical Medicine and Rehabilitation Research Center, School of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, Iran

[4] Department of Food Science and Technology, School of Nutrition and Food Sciences, Food Sciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Biotechnology Research Center, School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

[6] IT Department, School of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, Iran

Published online: 05 October 2017

Introduction

Bacterial vaginosis (BV) is the most common cause of vaginitis in women of reproductive age [1–3]. In BV, normal vaginal bacterial flora (Lactobacilli) changes, such that lactobacilli are reduced, vaginal pH is increased to more than 4.5, and the number of anaerobic bacteria (including Streptococci, *Gardnerella vaginalis*, *Mobiluncus*, Streptococci group B, and *Mycoplasma hominis*) is dramatically increased [4, 5].

Lactobacilli are the best known natural vaginal flora, and are capable of reducing pH and maintaining acidic medium of vagina (mainly due to acidic property of hydrogen peroxide enzyme). They also produce bacteriocins, which eradicate other bacteria [6, 7]. Bacteria and parasites such as trichomonas are infrequent where lactobacilli are dominant. On the other hand, where lactobacilli are scarce, infections such as bacterial vaginosis and also sexually transmitted diseases (such as gonorrhea, chlamydia, syphilis, trichomonas, HIV and human papilloma virus (HPV) that leads to cervical cancer) will dramatically increase [8, 9].

Sexual activity is among the main risk factors for BV [10, 11]. Most studies have also considered vaginal douche, smoking [12, 13], IUD, and antibiotics responsible for BV [14].

BV is usually treated with oral or vaginal administration of metronidazole or clindamycin. In some cases, treatment of BV fails due to medication side-effects or frequent recurrence of infection [15]. Recurrence of BV is a major problem in its treatment. Previous studies have shown that after BV treatment with metronidazole, 50–70% of women experience recurrence within 4–6 weeks and almost 70% within 90 days [16–18].

Prebiotics are indigestible food compounds that selectively stimulate or inhibit bacterial development and activity, and promote human health [19, 20]. Prebiotics are nutrients that are consumed by certain bacteria as a source of carbon, and thus can be added to the medium to boost bacterial development and survival [21]. The main effects of these products include reinforcement of immune system, and antioxidant, detoxification, and anti-inflammatory properties. These microbes increase absorption of minerals especially calcium and magnesium through gastrointestinal tract and alleviate risk of diabetes type II [22–24].

An in vitro study conducted by Rousseau et al. [25] to determine the effect of oligosaccharide on pathogenic microorganisms of three species of lactobacillus (crispatus, *L. jensenii*, and *L. vaginalis*) showed that lactobacilli and oligosaccharides are good choices for formulas used for the prevention of vaginal infections [25]. Coste et al. [26] conducted a study to determine the efficacy and safety of prebiotics in improving vaginal balance in people that had been previously treated for BV, and their results showed that treatment with prebiotic vaginal gel improves natural vaginal flora following treatment of BV, which ensures reduced risk of recurrence [26].

Few studies have been conducted on the use of prebiotics for development of vaginal lactobacilli [26]. Thus, given the prevalence of bacterial vaginosis (one of the main reasons for women's visiting gynecology clinics), medication side-effects, or frequent recurrence of infection (with failed medication therapy in some cases), and also their particular mechanism of effect, prebiotics have currently provided researchers with an appropriate theoretical perspective for treatment of BV. The present study was conducted to compare the effects of prebiotic vaginal gel plus oral metronidazole tablets and oral metronidazole tablets alone on treatment and recurrence of BV.

Materials and methods

The present triple-blind randomized clinical trial was conducted after obtaining permission from ethics committee of Tabriz University of Medical Sciences (ethics code: 1394/736) and registration in RCT site (IRCT: 215121721917N5) in 2015 on 100 18- to 45-year-old women attending Sadatmandi hospital in Robat-Karim town. The study inclusion criteria were 18–45 years of age, married, and proof of bacterial vaginosis based on Amsel and Nugent criteria, having one sexual partner, no menstruation while using the gel, and no use of vaginal douche in the last 48 h. The study exclusion criteria were pregnancy, breastfeeding, menopause, idiopathic vaginal bleeding, use of vaginal gels and antibiotics or immunosuppressive medications during 14 days leading to intervention, daily use of alcohol and smoking, use of anti-coagulative medications such as Coumadin and disulfiram, no other trichomonas or candida vaginal infections during the present study, no medically known diseases such as diabetes, blood dyscrasias, thyroid and liver diseases as disclosed by patient, and unwillingness to use prebiotic gel.

Using power and sample size calculation (PS) software, and assuming 30% increase in loss of clinical and laboratory signs due to intervention, and based on data from Bohbot et al. study [27] showing 66% improvement in clinical and laboratory signs of bacterial vaginosis using oral metronidazole on day 14, sample size was determined 44 patients for each group, which was increased to 50 patients taking into account the possibility of a 10% sample loss, making total number of participants 100 patients.

Data collection tools included a demographic and obstetrics questionnaire, and clinical examination and laboratory diagnosis checklists. In the present study, BV was diagnosed through clinical examination and microscopic criteria based on Amsel and Nugent criteria. By definition,

in Amsel method, having three out of four diagnostic criteria, including homogeneous and gray-colored vaginal discharge, fishy smell following addition of potassium hydroxide 10%, presence of clue cells (bacteria-covered squamous epithelial cells), and vaginal discharge pH > 4.5 suggest diagnosis of BV [28, 29]. According to Nugent scoring system, scores between 4 and 6 with clue cells, or between 7 and 10 without are regarded as positive [30].

The researcher visited the health center and assessed 18–45-year old, non-pregnant, non-lactating, married women attending for bacterial vaginosis diagnostic tests.

Assessment involved clinical examination using a speculum, assessment of discharge in terms of form, consistency, color and smell, measurement of pH using pH paper (Merck, Germany) with accuracy of 0.5, and taking a sterile swab of vaginal discharge and transfer onto a glass slide and addition of a drop of KOH to emit fishy smell (Whiff test), and finally, having three out of four Amsel criteria.

Laboratory assessment of Nugent criterion was performed in people with positive Amsel criterion. Some of the discharges were taken with a sterile swab and placed on a glass slide, fixed with a spray fixator, and transferred to the laboratory on the same day. After gram staining by a laboratory technician, who was blinded to the Amsel criterion results or grouping of patients, Nugent score and the presence of clue cells were determined. Intraobserver reliability was assessed by sending the first five slides to the laboratory under two different names (two samples were taken from the first 5 patients) and results were compared. Finally, eligible women were contacted on the phone and invited to gynecology clinic to take part and receive medication.

In a briefing meeting (group or individual), the study objectives and method and confidentiality of data were explained, and informed written consents were obtained to recruit 100 eligible women to complete a demographic questionnaire. Then, eligible women were equally assigned to intervention and control groups using random blocks of 4–6. To blind assignments, identical packages containing prebiotic and placebo vaginal gels (same color, smell, and shape) were put in sealed and numbered envelopes by a non-involved person in the selection of participants and collection of data. Envelopes were given to participants as they entered the study.

Participants in the intervention group used three 250 mg metronidazole tablets (one every 8 h) plus a 5 mg prebiotic vaginal gel applicator (Trifolium vag) with 5 mg dose of 2% gel per day, and the control group used three 250 mg metronidazole tablets (one every 8 h) plus a placebo vaginal gel applicator per day. Prebiotic gel was produced in the formulation laboratory of School of Pharmacy of Tabriz University of Medical Sciences, and contained 2% red clover extract (Trifolium), 10% inulin, and 10% fructo-oligosaccharide.

The participants were briefed on how to use and how to observe hygiene issues associated to the applicator. They were asked to avoid vaginal douche, or vaginal medications and antibiotic medications during treatment. Using condoms during sex was also recommended.

Treatment was defined as elimination of all Amsel criteria or one remaining out of four, and also score of 0–3 with clue cells or 4–6 without based on Nugent scoring system. The first follow-up was carried out 10 ± 1 days after treatment in the form of examination and assessment of Amsel and Nugent criteria, and patients were asked about their satisfaction with treatment and its side-effects. The second follow-up, aiming to investigate recurrence, was carried out 90 ± 1 days after treatment, and Amsel and Nugent criteria were assessed again. Food record-24 h was completed by intervention and control groups at entering the study, and participants completed this form at home (on two non-holiday and one holiday days) and returned it. Mean value of these 3 days was determined, and groups were compared in terms of intake of micro- and macro-nutrients, especially intake of fiber and antioxidants. Intake of nutrients in the two groups was controlled and compared. Data were analyzed in SPSS-16.

Results

The present study was conducted from February 28th to August 22nd 2016, when 607 women were assessed in terms of eligibility criteria (Fig. 1), of whom, 100 women that met inclusion criteria were assigned to intervention (50 women) and control (50 women) groups.

According to the results, mean (standard deviation) age at marriage was 19.28 (2.65) years in the control group, and 19.38 (2.6) years in the intervention group. Mean (standard deviation) of number of children was 1.86 (0.94) in the control group, and 2.14 (1.06) in the intervention group. Statistical tests showed no significant difference between the two groups in terms of personal and social characteristics (Table 1).

Comparing participants' clinical complaints before intervention showed no significant difference between intervention and control groups.

Comparing history of infection and treatment showed no significant difference between control and intervention groups in terms of infection during the last year ($P = 0.5$), or history of treatment of spouse ($P = 0.228$).

Comparing clinical examination and laboratory results before intervention showed vaginal pH > 4.5 in all participants, Whiff test was positive in all participants and clue cells were reported in 64% of women in the control group and 74% of women in intervention ($P = 0.194$). Lactobacillus count was less than one in 60% of women in the

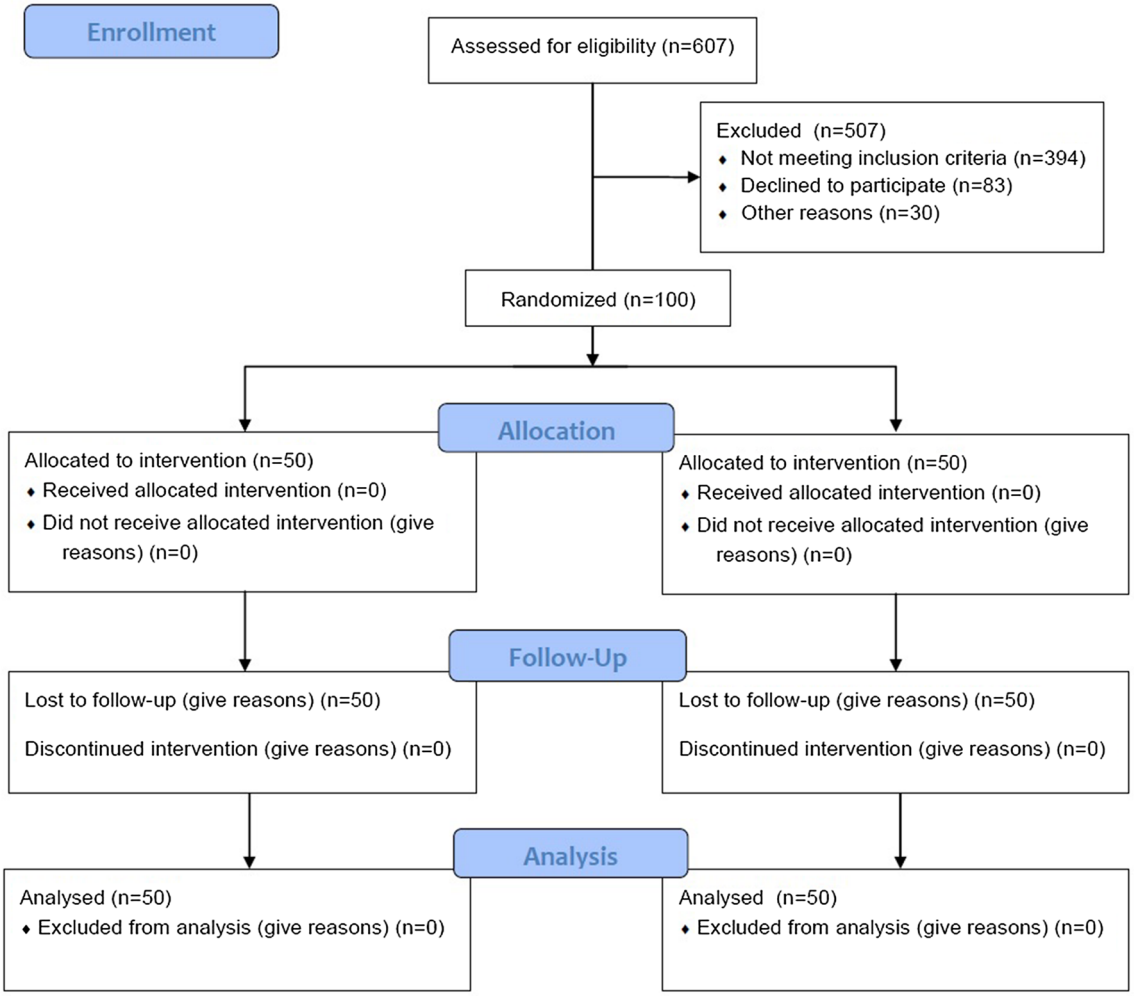

Fig. 1 Flowchart of study

control group and zero in 54% of those in intervention ($P = 0.236$). Mean (SD) Nugent score was 6.0 (1.0) in the control group and 5.8 (0.9) in intervention, with no significant difference between two groups ($P = 0.164$).

Table 2 shows clinical complaints on 10 ± 3 and 90 ± 3 days by groups. The results obtained showed that in the first stage, all complaints (except dyspareunia and itching) were significantly lower in the intervention group compared to control, and in the second stage, malodorous discharges ($P < 0.001$) and malodor during sex ($P = 0.001$) were significantly lower in the intervention group compared to the control group (Table 2).

Comparing participants' clinical examinations and laboratory results showed that vaginitis and cervicitis, vaginal discharge and positive Whiff test on 10 ± 3 day after intervention were significantly lower in the intervention groups compared to the control group. Vaginal pH less than 4.5 ($P < 0.001$) and positive clue cells ($P < 0.003$) were significantly lower in the intervention group compared to the control group (Table 3).

On 90 ± 3 day after intervention, significant differences were observed between the two groups in terms of vaginitis and cervicitis, vaginal discharge, Whiff test, and positive clue cells ($P < 0.001$), which meant that score of each of the above variables was significantly lower in the intervention group compared to the control group. Vaginal pH was less than 4.5 in all women in the intervention group ($P < 0.001$). Meanwhile, lactobacillus count was significantly lower in the intervention group compared to the control group in the range of 1–4 ($P = 0.002$) (Table 3).

The effects of treatment on Amsel and Nugent criteria on 10 ± 3 and 90 ± 3 days are shown in Table 4.

Comparing prebiotic gel and placebo groups in terms of satisfaction with treatment showed that 94% in the intervention group were satisfied and only 6% were dissatisfied,

Table 1 Personal and social details of participants in intervention and control groups

Personal details	Control group ($n = 50$) Number (%)	The intervention group ($n = 50$) Number (%)	P value
Age (years) Mean (SD)	33.48 (0.90)	34.52 (0.94)	0.430[a]
Education			
Primary and junior high	15 (30)	22 (44)	0.325[b]
Senior high and diploma	30 (60)	23 (46)	
University	5 (10)	5 (10)	
Occupation			
Housewife	37 (47.4)	41 (52.6)	0.334[b]
Mode of childbirth			
Vaginal	36 (78.3)	10 (21.7)	0.123[b]
History of abortion	7 (14)	7 (14)	1[b]
Type of contraception			
Hormonal	7 (14)	9 (18)	0.945[b]
IUD	7 (14)	5 (10)	
Condom	9 (18)	8 (16)	
Interrupted	23 (46)	23 (46)	
Tubal ligation in men and women	4 (8)	5 (10)	
History of vaginal infection	46 (92)	45 (90)	1[c]

[a]Independent *T* test

[b]Chi square

[c]Fisher's exact test

Table 2 Clinical complaints of participants by groups on 10 ± 3 and 90 ± 3 days after intervention

Complaint	Day 10 ± 3		P	Day 90 ± 3		P
	Intervention ($n = 50$) Number (%)	Control ($n = 50$) Number (%)		Intervention ($n = 50$) Number (%)	Control ($n = 50$) Number (%)	
Malodorous discharge	5 (10.0)	15 (30.0)	0.011[a]	6 (12.0)	32 (64.0)	< 0.001[a]
Dyspareunia	3 (6.0)	6 (12.0)	0.243[b]	2 (4.0)	0 (0)	0.247[a]
Suprapubic pain	2 (4.0)	3 (6.0)	0.021[b]	3 (6.0)	5 (10.0)	0.351[b]
Malodor during sex	3 (6.0)	12 (24.0)	0.011[a]	2 (4.0)	10 (2.0)	0.001[a]
Itching	0 (0)	2 (4.0)	0.247[b]	0 (0)	3 (6.0)	0.133[b]

[a]Chi square

[b]Fisher's exact test

and in the control group, 82% were satisfied and the remaining 18% were dissatisfied.

Comparing prebiotic gel and placebo groups in terms of side-effects showed that in prebiotic gel group, 30% reported suprapubic pain, 2% vaginal itching, 2% burning, 2% stomach ache and burning, and 1% burning and itching, and the remaining 60% reported no side-effects, and in placebo group, 82% reported no side-effects, and only 8% reported suprapubic pain, 8% vaginal itching, and 2% burning.

Discussion

The present clinical trial was conducted with the aim to determine the effect of prebiotic vaginal gel with oral metronidazole tablets on treatment and recurrence of bacterial vaginosis.

The present study results showed that use of prebiotic vaginal gel with oral metronidazole tablets in the intervention group compared to placebo vaginal gel with oral metronidazole tablets in the control group reduced clinical

Table 3 Participants' clinical examinations and laboratory results by groups

Criteria	10 ± 3 day		P^a	90 ± 3 day		P^a
	Intervention ($n = 50$)	Control ($n = 50$)		Intervention ($n = 50$)	Control ($n = 50$)	
	Number (%)	Number (%)		Number (%)	Number (%)	
Vaginitis	3 (6.0)	10 (20.0)	0.036	3 (6.0)	29 (58.0)	< 0.001
Cervicitis	8 (16.0)	24 (48.0)	0.001	2 (4.0)	21 (42.0)	< 0.001
Vaginal discharge	8 (26.0)	19 (38.0)	0.012	15 (30.0)	33 (66.0)	< 0.001
Whiff test	5 (10.0)	15 (30.0)	0.011	11 (22.0)	34 (68.0)	< 0.001
pH < 4.5	50 (100.0)	39 (78.0)	< 0.001	50 (100.0)	34 (68.0)	< 0.001
Positive clue cell	4 (8.0)	16 (32.0)	0.003	31 (62.0)	8 (16.0)	< 0.001
Lactobacilli count						
> 30	3 (6.0)	5 (10.0)	0.357	13 (26.0)	4 (2.0)	0.002
5–30	–	–		–	–	
1–4	47 (94.0)	45 (90.0)		37 (74.0)	48 (96.0)	
< 1	–	–		–	–	
Gardnerella count						
> 30	–	–	0.500	–	–	0.102
5–30	–	–		–	–	
1–4	47 (94.0)	46 (92.0)		–	–	
< 1	–	–		49 (98.0)	45 (90.0)	
Gram-negative bacilli count						
> 30	–	–	0.013	–	–	
5–30	–	–		–	–	
1–4	9 (18.0)	20 (60.0)		14 (28.0)	37 (74.0)	
Zero	41 (82.0)	30 (60.0)		36 (72.0)	13 (26.0)	

[a]Chi square

complaints and also treated BV based on Amsel and Nugent criteria.

Generally, the effect of prebiotics on the vaginal natural flora and pathogen has rarely been studied, even though they provide an interesting concept for improvement in the quality of vaginal flora. In the in vitro study, Rousseau et al. [25] addressed the effect of oligosaccharide on beneficial microorganisms such as three species of lactobacillus (crispatus, *L. jensenii*, and *L. vaginalis*). Vaginal discharge samples were taken from fifty 18- to 40-year-old-women with BV and placed in prebiotic medium containing oligosaccharide for 48 h. Prebiotic gels, including fructo-oligosaccharide (Raftilose and Actilight) and galactosaccharides with acidic binding were different. This test was effective in improving development of the three beneficial species of vaginal bacteria, but ineffective on pathogenic microorganisms of genitourinary tract including candida albicans, *E. coli*, and gardnerella vaginitis, which meant that fructo-oligosaccharide (Actilight) and galactosaccharides were effective only on development of lactobacilli, but pathogenic microorganisms were unable to metabolize prebiotics [25].

The results obtained in a clinical trial conducted by Coste et al. [26] with the aim to determine the efficacy and safety of prebiotic gel on BV in 42 participants, Coste showed that Nugent score was normal in the intervention group, but average or positive in 33% of those in the control group. After 16 days, Nugent score remained normal in the intervention group, but only 24% of those in the control group had normal scores. Moreover, vaginal flora and pH returned to normal in prebiotic receiving group [26].

We found a significant differences in Nugent scores, lactobacillus and gardenella colonies count on 10 ± 3 day after intervention between intervention and control groups, and also on 90 ± 3 day between intervention and control groups, which consistent with mentioned studies.

The results obtained showed that on day 10 ± 3, all complaints (except dyspareunia and itching) were significantly lower in the intervention group compared to the control group, and on day 90 ± 3, malodorous discharges and malodor during sexual intercourse were significantly lower in the intervention group compared to the control group. Emery et al. [31] conducted a study on 728 women to determine the effect of administration of vaginal lactose tablets on BV. Lactose was used as a vaginal prebiotic to support development of lactobacilli and synthesis of lactic acid as a new approach to treatment and prevention of

Table 4 Participants' clinical examinations and laboratory results by groups on 10 ± 3 and 90 ± 3 days after intervention

Variable	The intervention group			Control group			Day 10 OR, 95% CI	P^a	Day 90 OR, 95% CI	P^a
	Before intervention ($n = 50$)	Day 10 $n = 50$	Day 90 $n = 50$	Before intervention ($n = 50$)	Day 10 $n = 50$	Day 90 $n = 50$				
Treatment based on Amsel and Nugent criteria	–	38 (76.0)	42 (84.0)		15 (30.0)	31 (62.0)	4.3 (2.7–9.4)	0.012	3.7 (1.3–8.9)	< 0.001
Amsel criterion										
Absence of homogeneous discharge	–	42 (84.0)	44 (88.0)		31 (62.0)	18 (36.0)	3.8 (1.2–11.6)	0.017	13.0 (1.3–8.9)	< 0.001
Absence of clue cells	–	46 (92.0)	42 (84.0)		34 (68.0)	19 (38.0)	5.4 (1.6–17.6)	0.003	8.5 (3.3–22.9)	< 0.001
Negative Whiff test	–	45 (90.0)	39 (78.0)		35 (70.0)	16 (32.0)	3.8 (1.2–11.6)	0.017	7.5 (3.0–18.4)	< 0.001
pH < 4.5	–	50 (100)	50 (100)		39 (78.0)	34 (68.0)	8.8 (12.34–49.4)	< 0.001	6.9 (2.8–23.2)	< 0.001
Treatment based on Nugent criterion	–	43 (86.0)	48 (96.0)		29 (58.0)	37 (74.0)	8.4 (1.7–29.6)	0.001	4.4 (1.6–11.8)	0.002

[a]Binary logistic regression

overgrowth of vaginal pathogens. Vaginal discharges and their malodor were eliminated in 90% of women using prebiotic within 1 week. Vaginal itching and burning improved in 83% of women, and vaginal dryness in 76% 1 week after treatment [31]. In the present study, comparing side-effects in prebiotic gel and placebo groups showed that that in prebiotic gel group, 30% reported suprapubic pain, 2% vaginal itching, 2% burning, 2% suprapubic pain and burning, and 1% burning and itching,

Given the present study, further clinical trials are recommended on larger sample sizes of women with BV.

The study strengths and limitations

In the present controlled clinical trial, strong points included random allocation, blind allocation, and triple-blinding, evaluation of bacterial vaginosis based on both Amsel and Nugent criteria, and controlling food intake using a reminder form. Short-term follow-up (3 months) to assess recurrence of BV was a limitation of the present study.

Conclusion

Adjuvant treatment using prebiotic vaginal gel increased the efficacy of treatment of BV with oral metronidazole. Further studies with longer follow-up periods on this subject are recommended.

Acknowledgements This article was extracted from MSc thesis approved and funded by research deputy of Tabriz University of Medical Sciences. Thus, the researcher is obliged to the deputy, director of Sadatmandi health center affiliated to Iran University of Medical Sciences, and participants.

Compliance with ethical standards

Funding This study was funded by Tabriz University of medical sciences.

Conflict of interest We have no conflict of interest.

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

Ethical approval All procedures performed in studies involving human participants 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. This article does not contain any studies with human participants performed by any of the authors.

References

1. Anukam KC, Reid G (2007) Organisms associated with bacterial vaginosis in Nigerian women as determined by PCR-DGGE and 16S rRNA gene sequence. Afr Health Sci 7(2):68–72
2. Oduyebo OO, Anorlu RI, Ogunsola FT (2009) The effects of antimicrobial therapy on bacterial vaginosis in non-pregnant women. Cochrane Database Syst Rev 3:Cd006055
3. Verstraelen H, Verhelst R (2009) Bacterial vaginosis: an update on diagnosis and treatment. Expert Rev Anti-Infect Ther 7(9):1109–1124
4. Berek JS, Novak E (2012) Berek & Novak's gynecology. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, United States
5. Simoes JA, Bahamondes LG, Camargo RP, Alves VM, Zaneveld LJ, Waller DP, Schwartz J, Callahan MM, Mauck CK (2006) A pilot clinical trial comparing an acid-buffering formulation (ACIDFORM gel) with metronidazole gel for the treatment of symptomatic bacterial vaginosis. Br J Clin Pharmacol 61(2):211–217
6. Pavlova SI, Kilic AO, Kilic SS, So JS, Nader-Macias ME, Simoes JA, Tao L (2002) Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rRNA gene sequences. J Appl Microbiol 92(3):451–459
7. Tarnberg M, Jakobsson T, Jonasson J, Forsum U (2002) Identification of randomly selected colonies of lactobacilli from normal vaginal fluid by pyrosequencing of the 16S rDNA variable V1 and V3 regions. Acta Pathol Microbiol Immunol Scand 110(11):802–810
8. Donders GG (2007) Definition and classification of abnormal vaginal flora. Best Pract Res Clin Obstet Gynaecol 21(3):355–373
9. Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S (2001) Defense factors of vaginal lactobacilli. Am J Obstet Gynecol 185(2):375–379
10. Yen S, Shafer MA, Moncada J, Campbell CJ, Flinn SD, Boyer CB (2003) Bacterial vaginosis in sexually experienced and non-sexually experienced young women entering the military. Obstet Gynecol 102(5 Pt 1):927–933
11. Fethers KA, Fairley CK, Morton A, Hocking JS, Hopkins C, Kennedy LJ, Fehler G, Bradshaw CS (2009) Early sexual experiences and risk factors for bacterial vaginosis. J Infect Dis 200(11):1662–1670
12. Bradshaw CS, Walker SM, Vodstrcil LA, Bilardi JE, Law M, Hocking JS, Fethers KA, Fehler G, Petersen S, Tabrizi SN, Chen MY, Garland SM, Fairley CK (2014) The influence of behaviors and relationships on the vaginal microbiota of women and their female partners: the WOW health study. J Infect Dis 209(10):1562–1572
13. Schwebke JR, Desmond RA, Oh MK (2004) Predictors of bacterial vaginosis in adolescent women who douche. Sex Transm Dis 31(7):433–436
14. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M (2001) Bacterial vaginosis: a public health review. BJOG Int J Obstet Gynaecol 108(5):439–450
15. Jafarzadeh H, Ivanbagha R, Hasanzadeh D, Sorush Barhaghi MH, Sayyah Melli M, Mohamad Poorasl A (2011) Comparsion of the effect of lactobacillus acidophilus suppository and metronidazol vaginal tablet on bacterial vaginosis. Hayat 17(3):5–14
16. Gellar M, Nelson A (2004) Diagnosis and treatment of recurrent and persistent vaginitis. Womens Health Gynecol 4:137–146
17. Sobel JD, Ferris D, Schwebke J, Nyirjesy P, Wiesenfeld HC, Peipert J, Soper D, Ohmit SE, Hillier SL (2006) Suppressive antibacterial therapy with 0.75% metronidazole vaginal gel to prevent recurrent bacterial vaginosis. Am J Obstet Gynecol 194(5):1283–1289. doi:10.1016/j.ajog.2005.11.041
18. Wilson J (2004) Managing recurrent bacterial vaginosis. Sex Transm Infect 80(1):8–11
19. Prebiotics (2010). http://www.ncbi.nlm.nih.gov/mesh/?term=prebiotics. Accessed 07 Dec 2017
20. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6):1401–1412
21. Aghajani A, Pourahmad R, Mahdavi Adeli H (2011) The effect of prebiotics on probiotic yogurt containing *Lactobacillus casei*. J Food Technol Nutr 8(4):73–82
22. Hosseini Kangelani A (2013) Functional foods, prebiotics and probiotics. In: Paper presented at the National Congress of Food Science and Technology, Shiraz
23. Rastall RA (2010) Functional oligosaccharides: application and manufacture. Annu Rev Food Sci Technol 1:305–339
24. Roberfroid MB (2001) Prebiotics: preferential substrates for specific germs? Am J Clin Nutr 73(2 Suppl):406s–409s
25. Rousseau V, Lepargneur JP, Roques C, Remaud-Simeon M, Paul F (2005) Prebiotic effects of oligosaccharides on selected vaginal lactobacilli and pathogenic microorganisms. Anaerobe 11(3):145–153
26. Coste I, Judlin P, Lepargneur JP, Bou-Antoun S (2012) Safety and efficacy of an intravaginal prebiotic gel in the prevention of recurrent bacterial vaginosis: a randomized double-blind study. Obstetr Gynecol Int 2012:147867
27. Bohbot JM, Vicaut E, Fagnen D, Brauman M (2010) Treatment of bacterial vaginosis: a multicenter, double-blind, double-dummy, randomised phase III study comparing secnidazole and metronidazole. Infect Dis Obstet Gynecol 2010:1–6
28. Fethers KA, Fairley CK, Hocking JS, Gurrin LC, Bradshaw CS (2008) Sexual risk factors and bacterial vaginosis: a systematic review and meta-analysis. Clin Infect Dis 47(11):1426–1435
29. Spiegel CA, Amsel R, Holmes KK (1983) Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. J Clin Microbiol 18(1):170–177
30. Borghei MS, Saatian M, Mohammadyari F (2010) Diagnostic value of Pap smear test in screening of bacterial vaginosis. Med Sci J Islam Azad Univ 20(3):189–193
31. Emery S, Tomlinson M, Hansen I (2012) P51 patient-reported effects of vaginal lactose as a prebiotic for bacterial vaginosis. Sex Transm Dis 88(Suppl 1):A27