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The Effect of use of Vaginal Lactobacillus Rhamnosus for Prevention of Recurrence of Vulvovaginal Candidiasis: A randomized controlled trial

Alaa M. Ismail, M.D.,
Ahmed M. Abbas, M.D.,
Ahmed H. Shams MSc.,
Momen A. Kamel, M.D.,
Mohamed H. Makarem, M.D.

Department of Obstetrics and Gynecology, Woman's Health Hospital, Assiut University, Egypt

ABSTRACT

Objectives: The study aimed to investigate the role of vaginal administration of Lactobacillus Rhamnosus after vaginal miconazole for prevention of recurrence among women with vulvovaginal candidiasis (VVC).

Materials and Methods: A randomized clinical study was done in Women Health Hospital–Assiut University–Egypt. All women presented with symptoms suggestive of VVC to the clinic had been examined and approached for participation. Eligible participants were randomly assigned to one of three groups: Group (A) received lactobacillus containing vaginal capsules daily for one week postmenstrual. Group (B) received vaginal miconazole 400 mg once daily for 3 days at bedtime postmenstrual. Group (C) received vaginal miconazole 400 mg once daily for 3 days at bedtime postmenstrual followed by lactobacillus containing vaginal capsules twice daily for one week. The primary outcome was to study the rate of recurrence of symptoms after 1, 3, 6 months of treatment.

Results: During the study period, 202 participants with recurrent VVC were approached to participate in this study. No significant differences were found between the three study groups with regards patients' age, residence, parity. After 1 month, symptoms of VVC recurred in 68.4%, 24.6% and 17.2% of women in group A, B, C consecutively. The recurrence rate increased after 3 months to become 87.5%, 60% and 33.3% consecutively in the three groups. Finally after 6 months, 94.4%, 88.7%, and 44.6% of women in group A, B, C consecutively suffered from RVVC. The recurrence rate was lower in group C (combination group) with statistically significant difference ($p=0.0001$).

Conclusion: Vaginal administration of Lactobacillus Rhamnosus twice daily for 1 week after vaginal miconazole leads to vaginal colonization and associated at 6 months follow up with decrease the recurrence rate of VVC.

Keywords: Vaginal discharge, vulvovaginal candidiasis, probiotics, lactobacillus Rhamnosus, antifungal.

Correspondence to: Ahmed M. Abbas, M.D., Woman's Health Center, Assiut University, Assiut, Egypt, Telephone number: +20882414616. Cellular phone number: +2 01003385183., E-mail: bmr90@hotmail.com

Introduction

Yeast infections of the vagina are what most of the women perceive when they hear the term “vaginitis”. One of the species of fungus called *Candida* (C.) causes them. Vulvovaginal candidiasis (VVC) is one of the most frequent diagnoses in women attending genitourinary clinics. Of approximately 150 *Candidal* types, *Candida albicans* is by far the most common in gynecological infections; it can be found in approximately 80-90% of cases⁽¹⁾. It produces thick, white, odorless vaginal discharge with the consistency of cottage cheese⁽¹⁾. In a multicenter study, the prevalence of VVC was ranged between 29% and 49%⁽²⁾. In 40–50% of women; recurrent VVC, defined as four or more episodes every year, can be experienced⁽³⁾.

The normal vaginal microbiota is a dynamic system that continually fluctuates under the environmental changes and different physiological conditions. The vagina of a healthy fertile woman harbors an extensive number of bacteria. In 1894, the German Obstetrician and Gynecologist, Döderlein (1860-1941), isolated Gram-positive, catalase-negative rods, now referred to as *Lactobacillus*, from the vagina of healthy pregnant women⁽⁴⁾.

Recurrent VVC may be attributed to the elimination of the commensal organisms in the vagina by the antimicrobial thereby increasing susceptibility to recolonization by pathogens⁽⁵⁾. This is one of the main reasons for considering the use of probiotics, to replenish the commensal microbes as a way to lower the risk of reinfection. Probiotics are defined as live microorganisms which, when consumed in appropriate amounts, give a health benefit on the host⁽⁶⁾.

The basis for use of probiotics in vulvovaginal infection emerged in 1973 when healthy women with no history of UTI were reported to have lactobacilli in their vagina⁽⁷⁾.

Vaginal *Lactobacillus* species is capable of adhering to vaginal epithelial cells, persisting in the vagina, and that are capable of expressing anti-candida protective factors. Lactobacilli inhibit the growth of pathogenic microorganisms by several mechanisms including; production of lactic acid, hydrogen peroxide,

bacteriocins and bacteriocin-like substances, and competition with other microorganisms for adherence to the vaginal epithelium^(8,9).

The aim of the present study was to investigate the role of vaginal administration of *Lactobacillus Rhamnosus* after vaginal miconazole for prevention of recurrence among women with VVC.

Materials and Methods

The current study was a randomized controlled trial conducted in the outpatient Gynecology Clinic of Women Health Hospital, Assiut University between January 2014 and December 2014. All women presented with symptoms suggestive of VVC to the clinic had been examined and approached for participation. The Assiut University Medical Ethical Review Board approved the study.

Study participants:

We had included women fulfilled the inclusion criteria, which were married, aged between 18 and 45, premenopausal, signed informed consent and diagnosed as VVC infection, based on symptoms suggestive of VVC as vaginal soreness, burning, dyspareunia, pruritus and vaginal discharge typically cottage-cheese-like white discharge, confirmed by direct microscopic examination of vaginal secretions taken by sterile vaginal swab revealed presence of budding cells or pseudohyphae.

We had excluded pregnant, lactating women, those who had received any antimicrobial treatment in the last 2 weeks prior to recruitment, and those who refused to participate in the study.

Randomization:

Randomization was done using computer-generated random table. After acceptance of eligible women to participate in the study, they were assigned randomly to one of three groups.

Patients were randomized to receive either twice daily dose of *Lactobacillus* vaginal capsules for one week or once daily antifungal vaginal suppository for 3 days or both of them. Allocation concealment was done

using serially-numbered closed opaque envelope. Counseling for participation was done before recruitment. Once allocation had been done, it could not be changed.

Intervention

All eligible participants signed a written consent after reading the patient information sheet or having it read to them. Eligible participants were randomly assigned to one of three groups: Group (A) received lactobacillus containing vaginal capsules (1 billion lactobacilli per capsule) (gyniel-premium company®) twice daily for one week postmenstrual.

Group (B) received antifungal vaginal suppository containing miconazole 400 mg (gynozol-pharco company®) once daily for 3 days at bedtime postmenstrual.

Group (C) received antifungal vaginal suppository containing miconazole 400 mg (gynozol-pharco company®) once daily for 3 days at bedtime postmenstrual followed by lactobacillus containing vaginal capsules (1 billion lactobacilli per capsule) (gyniel-premium company®) twice daily for one week.

Follow-up schedule:

All participants were evaluated three times by the same gynecologist during the follow-up period; the first after 1 month, the second after 3 months and the third after 6 months from the end of treatment. Evaluation of improvement had been assessed by the disappearance of symptoms, and direct microscopic examination of a sample of vaginal discharge revealed an absence of budding cells or pseudohyphae. Any local vaginal side effects like burning or itching encountered during treatment were recorded. Moreover, any general side effects complained by the patients were also recorded.

Sample size calculation:

Sample size calculation was based on the primary outcome (the cure rate 6 months after treatment). Previous studies reported that women who had treated by lactobacillus rhamnosus once daily had only 30% cure rate after 60 days from the starting of the treatment while those treated by placebo once daily had cure rate

of 12%⁽¹⁰⁾. Using two-sided chi-square test with α of 0.05, a minimum sample size of at least 180 in the 3 groups. This will give 60 patients in each group, using 90% power and confidence interval 95% (Epi-info™, CDC, USA. 2008).

Data collection and analysis:

The data were collected and entered into Microsoft access database to be analyzed using the Statistical Program for Social Science (SPSS Inc., Chicago, version 18). Comparisons between the groups were done using ANOVA test to compare the mean values between groups in scale variables. However, chi-square test was used to compare the dichotomous and ordinal variables in the groups. Fisher exact test was used to compare between groups in case of abnormally distributed data. For analysis $p < 0.05$ was considered significant.

Results

Two hundred and two participants with recurrent VVC were approached to participate in this study. Twenty-two women have excluded: seven women were pregnant, five were lactating and eight had received antibiotic treatments in the prior week. Moreover, two women refused to participate in the study. The remaining 180 patients were randomly assigned to the three groups (Fig. 1).

Table 1 shows the demographic characteristics of the study participants. No significant differences were found between the three study groups with regards patients' age, residence, parity. Concerning contraceptive utilization, more than 50% of participants were not using any method of contraception at the time of the study. No statistically significant difference between women using contraception in the three groups.

Table 2 shows the recurrence of symptoms after one, three and six months after the treatment with a significant statistical difference were found between the three study groups ($p=0.0001$). After follow-up, recurrence of symptoms after one and 3 months occurred commonly in Gyniel group more than

antifungal group followed by the combination group. The same observed after six months. No local or general side effects reported by the patients during the period

of treatment in all groups. The same observed after six months. No local or general side effects reported by the patients during the period of treatment in all groups.

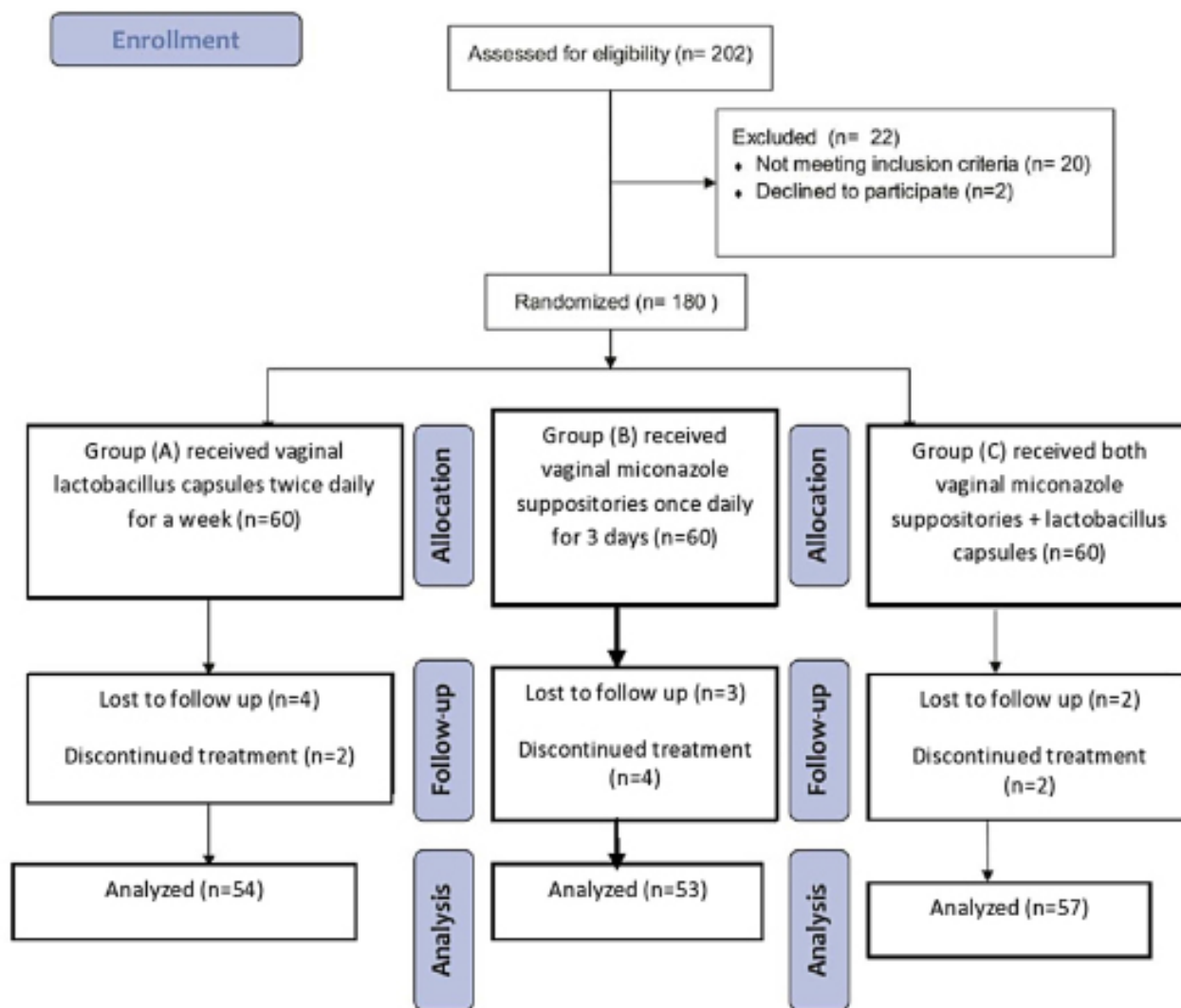


Fig. 1. The study flowchart.

Table 1. Demographic characteristics and contraceptive use among the study participants.

Variables	Group A (n= 60)	Group B (n= 60)	Group C (n= 60)	P
Age (mean \pm SD) [§]	34.47 \pm 7.63	34.95 \pm 8.24	34.50 \pm 7.73	0.227
Residency*				
Rural (n, %)	25 (41.7%)	21 (35.0%)	23 (38.3%)	0.754
Urban (n, %)	35 (58.3%)	39 (65.0%)	37 (61.7%)	

Table 1. Demographic characteristics and contraceptive use among the study participants. (Cont.)

Variables	Group A (n= 60)	Group B (n= 60)	Group C (n= 60)	P
Parity (mean \pm SD) [#]	3.98 \pm 2.60	3.87 \pm 2.43	3.62 \pm 2.28	0.414
Living children (mean \pm SD) [#]	3.78 \pm 2.57	3.72 \pm 2.16	3.43 \pm 2.22	0.406
Abortions (mean \pm SD) [#]	0.60 \pm 1.01	0.75 \pm 1.27	0.75 \pm 1.26	0.727
Contraception use *				
Hormonal	13 (21.7%)	11 (18.3%)	16 (26.7%)	0.477
Non hormonal	17 (28.3%)	14 (23.3%)	17 (28.3%)	0.356
None	30 (50%)	35 (58.4%)	27 (45%)	0.127

Group A: patients received Gyniel, Group B: patients received antifungal, Group C: patients received both of them.

[§] ANOVA test was used to compare between means as tested data was normally distributed

* Chi-square test was used to compare between percentages

[#] Fisher exact test was used to compare between means as tested data was not normally distributed

Table 2. The recurrence of symptoms after one, three and six months in the study groups.

	Group A			Group B			Group C		
	One month	Three months	Six months	One month	Three months	Six months	One month	Three months	Six months
	(n=57)	(n=56)	(n=54)	(n=57)	(n=55)	(n=53)	(n=58)	(n=57)	(n=56)
Recurrence of symptoms, n	39	49	51	14	33	47	10	19	25
%	68.4	87.5	94.4	24.6	60	88.7	17.2	33.3	44.6

Group A: patients received Gyniel, Group B: patients received antifungal, Group C: patients received both of them.

Chi-square test was used to compare between data, (P=0.0001 between the data after 1, 3, and 6 months)

Discussion

In the present randomized controlled trial we have studied the effect of an antifungal suppository containing miconazole 400 mg once daily for 3 days postmenstrual followed by lactobacillus containing vaginal capsules (1billion lactobacilli per capsules) twice daily for one week on prevention of recurrent VVC. To the best of our knowledge, there are no enough studies about the efficacy of combined local antifungal therapy plus lactobacillus containing vaginal capsules so the idea of our study is to prove if combined treatment will decrease the recurrence rate of VVC.

Probiotics such as lactobacillus were the most commonly recommended complementary therapy in

VVC⁽¹¹⁾. The supposed mechanism is that increasing the lactobacilli “good bacteria” will reduce harmful effects of other commensal organisms, such as *Candida* species⁽¹²⁾. *Lactobacillus*, which lowers the vaginal pH, has been shown to be associated with a decrease in *Candida* species colonization⁽¹³⁾.

In the present study we found a significant decrease in the recurrence rate of the combined therapy group (83.3% cure) compared to the other groups after one month (p=0.0001). This coincided with Ehrstrom et al (2010) who used vaginal capsules containing (*L. gasseri* LN40, *L. fermentum* LN99, *L. casei* subsp. *rhamnosus* LN113 and *P. acidilactici* LN23) for five days after treatment of VVC. One month later, the cure rate was 78%⁽¹⁴⁾.

After 3 months, we found also a significant decrease in the recurrence rate of the combined therapy group (66.7% cure) compared to the other groups ($p=0.0001$). This coincides with Murina et al (2014) whose patients were given 200 mg of fluconazole orally for 3 alternate days then slow-release vaginal tablets containing at least 0.4 billion live cells of each of *L. fermentum* LF10 and *L. acidophilus* LA02 for 10 consecutive nights. After 10 weeks, 86.0% of patients remained free of clinical recurrence⁽¹⁵⁾.

Also, there was a significant decrease in the recurrence rate of the combined group therapy (55.4% cure) compared to other groups after six months ($p=0.0001$). These results were not as good as those of Ehrstrom et al (2010) in which there was only 9% of women were still colonized by *Candida* six months after administration of probiotics⁽¹⁴⁾. Also, Murina et al (2014) found that 85.7% of patients remained free of symptoms for 7 months after combined treatment with oral fluconazole then vaginal *L. fermentum* LF10 and *L. acidophilus* LA02⁽¹⁵⁾.

We consider many positive points in our study. Our study revealed a simple line of treatment with promising results. This treatment is Gyniel which is cheap and available in the market. This treatment is natural with no major side effects. Furthermore, the treatment can be taken vaginally and it does not need special follow up or hospitalization.

However, it was difficult to take vaginal swabs for culture as it was costly; the study was difficult to expand because follow-up of the patients was difficult. Patients were lost to follow-up (about 10%) and this may be attributed to their rural origin and the long distances between their residency and our institute.

In conclusion, vaginal administration of *L. Rhamnosus* twice daily for 1 week after vaginal miconazole leads to vaginal colonization and associated at 6 months follow up with decrease the recurrence rate of VVC. We suggest that probiotics are cheap, available and noninvasive promising choices for treatment of cases of recurrent VVC. However, further studies are required support this recommendation in a larger number of patients.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Sobel JD. Vulvovaginal candidosis. *Lancet* 2007;369:1961-71.
2. Foxman B, Muraglia R, Dietz JP, Sobel JD, Wagner J. Prevalence of Recurrent Vulvovaginal Candidiasis in 5 European Countries and the United States: Results From an Internet Panel Survey. *J Low Genit Tract Dis* 2013;17:340-45.
3. Foxman B, Marsh Jv, Gillespie B, Sobel Jd. Frequency and response to vaginal symptoms among white and African American women. *Am J Public Health* 1998;80:150-3
4. Döderlein A. Das Scheidensekret und seine Bedeutung für das Puerperalfieber. Leipzig: Verlag von Edu Besardold. 1892.
5. Hooton TM, Hillier S, Johnson C, Roberts PL, Stamm WE. *Escherichia coli* bacteriuria and contraceptive method. *J Am Med Assoc* 1991;265:69.
6. Champagne Cp, Ross Rp, Saarela M, Hansen Kf, Charalampopoulos D. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *Int J Food Microbiol* 2011;149:185-93.
7. Imirzalioglu C, Hain T, Chakraborty T, Domann E. Hidden pathogens uncovered: metagenomic analysis of urinary tract infections. *Andrologia* 2008;66:71-40.
8. Barbes C, Boris S. Potential role of lactobacilli as prophylactic agents against genital pathogens. *AIDS Patient Care STDS* 1991;13:747-51.
9. Melis GB, Ibba MT, Steri B, Kotsonis P, Matta V, Paoletti AM. Role of pH as regulator of vaginal physiological environment. *Minerva Ginecol* 2000;111:121-52.
10. Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, Bruse AW. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immun Med Microbiol* 2003;35:131-4.
11. Watson CJ, Pirota M, Myers SP. Use of complementary and alternative medicine in recurrent vulvovaginal candidiasis—results of a practitioner survey. *Complement Ther Med* 2012;20:218-21.
12. Pirota M, Gunn J, Chondros P, Grover S, O'Malley P, Garland S. The effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomised controlled trial. *BMJ* 2004;329:458-64.
13. McClelland RS, Richardson BA, Hassan WM, Graham SM, Kiarie J, Baeten JM. Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. *J Infect Dis* 2009;199:1883-90.
14. Ehrström S, Daroczy K, Rylander E, Samuelsson C, Johannesson U, Anzén B, Pålsson C. Lactic acid bacteria colonization and clinical outcome after probiotic supplementation in conventionally treated bacterial vaginosis and vulvovaginal candidiasis. *Microbes Infect*

2010;12:691-99.

15. Murina F, Graziottin A, Vicariotto F, De Seta F. Can *Lactobacillus fermentum* LF10 and *Lactobacillus*

acidophilus LA02 in a slow-release vaginal product be useful for prevention of recurrent vulvovaginal candidiasis. *J Clin Gastroenterol* 2014;1:102-5.