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The role of vaginal *Lactobacillus Rhamnosus* (Normogin®) in preventing Bacterial Vaginosis in women with history of recurrences, undergoing surgical menopause: a prospective pilot study

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Abstract. – BACKGROUND: Bacterial vaginosis (BV), a poly-microbial clinical syndrome, is the most common cause of vaginal symptoms among women. The recurrence rate of BV is up to 30% after traditional antimicrobial therapy. *Lactobacillus rhamnosus* vaginal tablets have demonstrated to be a reliable topical effective and safe treatment to reduce the BV recurrence rate.

AIM: to assess topical long-lasting (6 months) *Lactobacillus rhamnosus* effectiveness in decreasing recurrences in women with positive anamnesis of recurrent BV and concomitant hypo-estrogenism as consequence of surgical menopause.

PATIENTS AND METHODS: A total of 22 consecutive patients affected by recurrent BV and treated for surgical menopause for benign pathology were enrolled. All women were treated with *Lactobacillus rhamnosus* vaginal tablets (Normogin®) according to the following protocol: 1 tablet/day for 6 days, than two tablets per week for 2 months and then one tablet once a week till 6 months.

RESULTS: Of the 22 women enrolled only one has been lost after the first visit. A total of 21 cases were reported; 7 out of 21 had only one case of recurrence, while 2 out of 21 had two episodes of BV during the year successive to menopause. No side effects have been reported.

CONCLUSIONS: Considering the low recurrence rate of BV during follow-up it seems that long-lasting treatment with vaginal tablets of *Lactobacillus rhamnosus* could reduce the BV recurrence also in women at high risk with positive history of pathology and undergoing surgical menopause with a safe profile. This study supports the use of vaginal *Lactobacillus rhamnosus* administration in high risk population without side effects.

Key Words:
Bacterial vaginosis recurrences, *Lactobacillus Rhamnosus*, Surgical menopause.

Introduction

Reproductive tract infections are the most common gynaecological complaints and represent a topic issue in modern clinical care. Bacterial vaginosis (BV), vulvovaginal candidiasis, and trichomoniasis are regarded as the most frequent vaginal infections[1].

The healthy vaginal flora is a balanced ecosystem characterized by various species of Lactobacilli, which plays an important protective role against genital infections. In women the depletion of vaginal Lactobacilli, leads to an overgrowth of diverse aerobic, anaerobic and microaerophilic species[2,3].

Frequency of the most common infectious agents for vaginitis ranges between 8-75% for *Gardnerella (G.) vaginalis*; 2-30% for *Candida albicans*; 1-34% for *Trichomonas vaginalis*[4,5].

BV is usually characterized by vaginal discharge, vaginal itching, irritation, or malodour, but in about 50% of cases BV appears as asymptomatic[6,8].

Diagnosis of BV is based on clinical criteria described by Amsel et al[9] and even on microscopic assessment using the Nugent score.

The four Amsel et al[9] criteria are the presence of a thin, white homogeneous discharge, presence of clue cells on microscopy, vaginal fluid pH > 4.5 and release of a fishy odor on addition of alkali (10% KOH). At least three of the four criteria should be present for a confirmed diagnosis[9].

The Nugent score is derived from microscopic assessment of a Gram-stained vaginal smear and the relative proportions of bacterial morphotypes are estimated, yielding a score between 0 and 10[10]. If the score is more than 7 is considered indicative of BV.

Treatment with metronidazole and clindamycin, administrated orally or intra-vaginally, have been shown to be effective treatments for BV by eradicating pathogen microorganism, but its long-term efficiency is often limited by relapse, most likely due to an inability to re-establish the normal *lactobacillus*-dominated vaginal flora[11].

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Some *in-vitro* studies were performed to elucidate the protective role of l*actobacillus (L.)* against genital infections. Lactobacilli protect vaginal epithelial through three different activities.

As first they produce lactic acid as a by-product of glycogen metabolism in the cell of the vaginal vault, thus acidifying the healthy vagina to a pH of 4.0-4.5, a level at which many pathogenic microbes cannot flourish. Furthermore, many species of *lactobacillus* produce hydrogen peroxide, which inhibit microbial growth[12,13]. The lactic acid of lactobacilli, which is mainly responsible for the low vaginal pH, contributes, probably even more than production of H_2O_2, to the inhibition of growth of *Gardnerella (G.) vaginalis*[14]. Third lactobacilli compete with pathogenic microorganism for adherence on epithelial cells[15]. Specific strain of lactobacilli are able to co-aggregate with *G. vaginalis* and block the adherence and/or displace previously adherent strains of *G. vaginalis* from vaginal epithelial[13].

In fact, oral administration of *L. acidophilus*, *L. rhamnosus* GR-1 and *L. fermentum* RC-14 for 2 months has been found to restore the normal vaginal microbiota, and it has shown to be more effective than placebo in preventing recurrence of BV and/or increasing vaginal colonization with lactobacilli[16-17].

In the vaginal microenvironment *L casei var-rhamnosus* spp is the only capable to survive up to seven weeks after exogenous implantation, determining positive clinical prevention in urogenital tract infections[18,21].

Recently, a long lasting therapy with *Lactobacillus rhamnosus* vaginal tablets has been proposed from Marcone et al[22,23] in women affected by BV and it has been shown a statistically significant decrease of the recurrence rate of BV after the recommended treatment with metronidazole.

Estrogens encourages the vaginal colonisation with lactobacilli, which metabolise glycogen to produce lactic acid and maintain a low vaginal pH that inhibits the growth of many pathogens.

In fact, in menopausal women, the gradual loss of glycogen and lactobacilli due to decline in circulating estrogens may cause an increase in vaginal pH[24,25].

For these reasons patients undergoing surgical menopause are at risk of BV, mainly if they presented positive history of symptomatic recurrence[26].

Aim of this study was to assess the effectiveness of a long lasting *Lactobacillus rhamnosus* vaginal tablets application in colonising the vagina and in preventing BV (Amsel criteria) recurrence rate in such high risk women.

Patients and Methods

A total of twenty-two women with positive history of symptomatic recurrent BV (positive Amsel criteria) confirmed by microbiological criteria (vaginal swabs collection with a Nugent score between 7 and 10) and undergoing surgical menopause for benign pathology were enrolled to this observational pilot clinical trial.

Regarding type of surgery, 15 of 22 women were treated for laparotomic bilateral annesiectomy and isterectomy surgery for different pathologies: 11 suffered from uterine miomas, 2 from uterine fibromatosis and pelvic inflammatory disease (PID) and 2 from endometrial hyperplasia; 7 of 22 women underwent a laparoscopic bilateral annesiectomy for ovarian cystis (Table I).

According to anamnestic relapses of BV, 5 out of 22 suffered 1-2 bacterial vaginosis recurrences per year, 11 of them had from 2 to 4 BV per year and 6 patients reported more than 4 recurrences/year (Table II).

In five out of twenty-two patients a positive history of cytological diagnosis of Human Papilloma Virus (HPV) dysplasia was concomitant.

Participants assigned to the intervention, after giving written informant consensus for surgical and clinical treatment, approved by our Ethical Committee, received a vaginal tablet containing *Lactobacillus rhamnosus* (Normogin®; Baldacci, Pisa, Italy) continuously for 6 days immediately after the surgical treatment, then two tablets weekly for 8 weeks and continued the treatment with one tablet per week for 6 months.

Each vaginal tablet contained at least 10^6 colony-forming units (CFU) of live and liophilized *L. rhamnosus.*

Follow-up were fixed at four weeks, 12 weeks and 24 and 48 weeks after surgery.

Table I. Indication to bilateral annesiectomy surgery.

Indication to surgery	Number of cases (n = 22)
Uterine mioma	11
Uterine mioma and PID	2
Endometrial hyperplasia	2
Ovarian cysts	7

Table II. Anamnestic incidence of BV.

Recurrence of bacterial vaginosis	Number of cases (n = 22)
1-2 episodes/year	5
2-4 episodes/year	11
> 4 recurrence/year	6

Microbiological diagnosis of BV (Nugent score) was performed in case of symptomatic occurrence (Amsel criteria).

In case of recurrence, metronidazole therapy was performed for 7 days and prophylaxis with vaginal lactobacilli continued till the final follow-up visit.

Recurrences of BV in general is estimated between 50% and 60%. Nevertheless, in literature, no data are available about the frequency of recurrences in high risk patients as those of our trial. For this reason this observational pilot study was set in order to design an randomized controlled trial (RCT), using experimental results to calculate power of the study.

Results

Age of population ranged between 46 and 51 years old, with an average of 48.1 years.

During a follow-up period of one year after surgery, one patient was lost after the first scheduled visit.

Twelve out of 21 women did not have any recurrence in the following 12 months while nine out them had 1 or more episodes of relapses (Table III).

A total of 11 BV were reported. Seven out of nine patients had only one case of recurrence, while two of them had two recurrences.

No side effects have been reported by the patients or by the gynecologists during the treatment period and during the study observation period.

Table III. Recurrence of BV.

Follow up (48 weeks)	Number of cases (n = 22)
≥ 1 episodes of BV	9
No episodes	12
Lost of follow up	1

Discussion

Vaginitis is the most common gynaecologic disorder in the primary care setting. In approximately 90 percent of affected women, this condition occur secondary to bacterial vaginosis, vulvovaginal candidiasis or trichomoniasis.

A healthy vagina normally contains many microorganisms; *Lactobacilli*, particularly hydrogen peroxide-producing species, appear to help prevent other vaginal microorganisms from multiplying to a level where they cause symptoms[2].

Although the aetiology of bacterial vaginosis is not completely understood, research has confirmed that BV is a synergistic, polymicrobial infection characterized by a shift in the bacterial flora of the vagina. Normal vaginal flora consists predominantly of lactobacilli, a natural host defence by producing both lactic acid, which maintains the vaginal pH below 4.5, and hydrogen peroxide, which inhibits the growth of non-catalase producing microorganisms. This combination creates a potent defence against the overgrowth of other vaginal bacteria, particularly anaerobes.

It has been observed that 70 to 95% of lactobacilli present in the vaginal flora of healthy women produce hydrogen peroxide. This percentage for women affected by BV drops to 5%.

Postmenopausal women are more susceptible to BV, a condition that can lead to other adverse health events such as urinary tract infections. Many postmenopausal women harbor a microbial consortium consistent with BV but without the induction of symptoms. Indeed, many potential pathogens (such as *Gardnerella vaginalis* and *Mobiluncus*) in the vaginal tract can exist as commensal inhabitants[27].

Metronidazole is the first-line treatment for bacterial vaginosis. However, oral metronidazole is associated with adverse effects such as gastrointestinal symptoms, unpleasant taste, and a disulfiram-like effect with alcohol ingestion and should not be used during pregnancy[28], with the risk of recurrence of BV[29].

The dominance of lactobacilli in healthy vaginal microbiota and its depletion in bacterial vaginosis (BV) has given rise to the concept of oral or vaginal treatment of probiotic *Lactobacillus* strains for the management of this condition[30,31].

Rossi et al[32] rating for the effectiveness of a long-term (24 months) intravaginal treatment with *Lactobacillus rhamnosus* on the vaginal pH and on the clinical symptoms in a group of 40

women affected by BV diagnosed by the Amsel criteria. They show that long-term administration of vaginal tablets containing *Lactobacillus rhamnosus* represents an effective and safe treatment for restoring the physiological vaginal pH and controlling BV symptoms. *L. rhamnosus* GR-1 was found to be the best of a group of 34 *Lactobacillus*[33].

In our study we observed a population at high risk of developing recurrent BV receiving *Lactobacillus rhamnosus* for a period of six month.

Fifty-seven point one percent of patients treated with Normogin® did not recur in the follow-up period, while 42.9% presented one or more episodes of BV.

Our results demonstrated a positive prevention efficacy with a long-term maintenance treatment with the aim to reduce the relapse.

A good compliance with the treatment was reported by most of patients, without side effects.

One limitation of this study is that we did not obtain vaginal swabs for assessment of the vaginal flora before administration of lactobacilli.

Conclusions

We can affirm that *Lactobacillus rhamnosus* has a prominent role in controlling the symptoms of BV and especially in the long term reduces the recurrence rate.

Conflict of Interest

None to declare.

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