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The influence of probiotics on the cervical malignancy diagnostics quality

Uticaj probiotika na kvalitet dijagnostike maligniteta cerviksa

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Abstract

Background/Aim. Probiotics help to provide an optimum balance in the intestines. Probiotics species competitive block toxic substances and growth of unwanted bacteria and yeast species while they compete for the space and food. Lactogyn® is the first oral probiotics on Serbian market dedicated to maintaining a normal vaginal flora. Lactogyn® contains two well studied probiotics strains – *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. Both of them are considered as probiotic agents with therapeutic properties increase the population of beneficial *lactobacillus* organisms within the vagina. The aim of this study was to exam an influence of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on results of cervical smear cytological testing including detection of atypical cells, detection of false positive and false negative findings as well as on vaginal microflora content in patients with vaginal infection signs and symptoms. **Methods.** Totally 250 women with signs of vaginal infection were selected to participate in the study. The study group comprised 125 patients taking studied probiotic strains along with specific anti-infective therapy. The control group comprised, also, 125 patients taking anti-infective agents, only. Probiotic preparation (Lactogyn® capsules) was administered orally (one capsule daily) during 4 weeks. Before and six weeks after beginning of the therapy a cervical smear cytological test (the Papanicolaou test), as well as microbiological examination of the vaginal smear were performed. **Results.** Number of cases of inflammation and atypical squamous cells of undetermined significance (ASCUS) in the study group were significantly higher before administration of the probiotic preparation. The number of lactobacilli was significantly higher, and the number of pathogenic microorganisms lower in the group treated with this preparation. **Conclusion.** The application of probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 concomitantly with specific anti-infective agents provides more reliable cytological diagnostics, reduces the number of false positive and false negative findings on cervical malignancy and normalizes vaginal microflora in higher percentage of patients with vaginal infections comapred with therapy including anti-infective agents only.

Key words:
probiotics; lactobacillus rhamnosus; lactobacillus reuteri; administration, oral; vaginal smears; treatment outcome.

Apstrakt

Uvod/Cilj. Probiotici pomažu u uspostavljanju ravnoteže u crevima. Probiotičke vrste kompetitivno inhibišu stvaranje toksičnih supstanci i rast manje poželjnih vrsta boreći se za prostor i hranu. Lactogyn® je prvi oralni probiotik registrovan u Srbiji za zaštitu zdravlja vaginalne flore. Ovaj preparat sadrži dve dobro poznate probiotske bakterije: *Lactobacillus rhamnosus* GR-1 i *Lactobacillus reuteri* RC-14. Cilj ove studije bio je da ispita uticaj primene *Lactobacillus rhamnosus* GR-1 i *Lactobacillus reuteri* RC-14 na rezultate citoloških testova koji se koriste u dijagnostici maligniteta cerviksa uključujući detekciju atipičnih ćelija, detekciju lažno pozitivnih i lažno negativnih nalaza, kao i uticaj na vaginalnu mikrofloru. **Metode.** U studiju bilo je uključeno 250 žena sa znacima vaginalne infekcije. Studijsku grupu činilo je 125 žena kod kojih je započeta primena specifične antiinfektivne terapije i probiotskog preparata (Lactogyn® kapsule, 1 kapsula dnevno), a kontrolnu grupu, takođe, 125 žena koje su bile na odgovarajućoj antiinfektivnoj terapiji, ali bez dodatog probiotskog preparata. Terapija je trajala 4 nedelje. Pre početka terapije, kao i 6 nedelja kasnije izvršeno je citološka i mikrobiološka analiza analiza cervikalnog brisa. **Rezultati.** U studijskoj grupi broj slučajeva sa inflamacijom i atipičnim skvamoznim ćelijama neodređene značajnosti (ASCUS) bio je značajno veći pre upotrebe probiotika. Broj laktobacila bio je značaj-

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no već, a broj patogenih mikroorganizama u vaginalnom sekretu niži u grupi žena koja je koristila preparat sa probiotskim bakterijama u odnosu na one koje su bile samo na terapiji antiinfektivnim agensima. **Zaključak.** Primena preparata na bazi probiotskih bakterija *Lactobacillus rhamnosus* GR-1 i *Lactobacillus reuteri* RC-14, u kombinaciji sa specifičnim antiinfektivnim agensima, kod žena sa znacima vaginalne infekcije, omogućava pouzdaniju citološku dijagnostiku, smanjuje procenat lažno negativnih i lažno pozitivnih nalaza i procenat nezadovoljavajućih i granično zadovoljavajućih nalaza na malignitet i normalizuje vaginalnu mikrofloru u značajno većem broju slučajeva nego što se postiže primenom samo antiinfektivnih agenasa.

Ključne reči:
probiotici; lactobacillus rhamnosus; lactobacillus reuteri; peroralna primena; vaginalni brisevi; lečenje ishod.

Introduction

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". One of the first researches in the field of probiotics were done by Nobel prize winner Elie Metchnikoff, Russian microbiologist, during an early 20th century (1905). Metchnikoff himself introduced in his diet sour milk fermented with the bacteria he called "Bulgarian Bacillus". The term "probiotics" was first introduced in 1953 by Kollath. Nowadays, interest in probiotics is growing at great speed. In recent years, more than 3.000 studies were published. There are numerous studies conducted with an aim to determine the effects of probiotics on the gastrointestinal tract and urogenital health of women; also, there are other studies which would try to discover other beneficial effects of probiotics on human health and animals. One of probiotics which were the most in focus of the scientists is *Lactobacillus rhamnosus* GG. It was isolated by scientists Goldin and Gorbach from the human digestive tract during 1985. The strain was named later with their initials (LGG). Compared to many pharmaceutical agents, probiotics are well tolerated and extremely safe, and serious adverse effects rarely occur [1, 2].

When urogenital health of women is in question, literature shows that for over 30 years, urologists have recognized in females that urinary pathogens almost always infect the host through ascension from the rectum, vagina to the urethra and bladder. Likewise, the *Lactobacillus* organisms that predominate in the vagina of healthy women, spread from the rectum and perineum and form a barrier in the vagina to the bladder entry by uropathogens. The number and types of microbes change due to sexual contact, hormone levels, diet, and so on. The concept of artificially boosting the lactobacilli number through probiotic instillation has been long conceived, but only in recent years it has be shown to be possible. Not all lactobacilli are effective, and to date clinical efficacy only exists for *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* B-54 and RC-14 [3, 4].

Lactogyn® is the first oral probiotic for restitution of vaginal flora on Serbian market. Lactogyn® capsule contains two patented and clinically proved probiotics strains – *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. Tingling and itching in the vaginal area and vaginal discharge, are complains known to many women. These symptoms are usually related to unbalanced vaginal microflora [5–7]. Lactogyn® can restitute vaginal flora balance and therefore may help, in a natural way, to establish and maintain urogenital health in women [8–11].

The presence of vaginal infections can deteriorate cytological diagnostics of malignacies when cervical smear is used. Due to infection, numerous microorganisms, white blood cells and degradatin products can be found. In order to improve the realibility of malignancy diagnostics, the treatment of the infections is necessary. After the successful treatment of the infections, the reinfections are frequient due to the disturbance of vaginal flora. The suplementation of lactobacilli as an important part of natural vaginal flora is necessary.

The goal of this study is to exam an influence of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on cervical smear diagnostics including: reliability of atypical cells detection, detection of false positive and false negative findings, as well as an influence on composition of vaginal microflora.

Methods

In this study 250 women who had vaginal discharge, burning and itching were included. Colposcopic examination was performed. Cervical smear was transferred to microscopy glass, fixed in 96% ethanol and tinted by the method of Papanicolaou (hematoxylin, methylorange, polychrome). After this, preparations were analyzed for malignancy and microorganisms by microscopic examination. To all patients the therapy for infections was prescribed and thus they were divided into two groups with 125 women each: the study group treated in addition to the specific antiinfective therapy with one capsule a day of Lactogyn® given perorally ("Jadran" galenska laboratorija, Rijeka, Croatia) containing probiotic bacteria *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* B-54 and RC-14 for 4 weeks, and the control group treated only with specific antiinfective therapy. After 6 weeks colposcopic examination was performed, was taken cervical smear and analyzed for malignancy and presence of microorganisms (bacteria, fungi, trichomonas). Parameters compared between these groups were signs (vaginal discharge), the presence of lactobacilli in the cervical smear and the malignancy finding.

Accuracy of the Papanicolaou test interpretation is a subjective based on experience of the screening cytotechnologist or pathologist; in good labs, error rate–false negative ranged from 5% to 15%. This test was done during a

routine pelvic exam of subjects enrolled in the study by scraping cells from the cervix prior and after the intervention. In this study, cells obtained from the uterine cervix and endocervix were sampled, put on a glass slide, stained, and interpreted by gynecologist-pathologist. The follow-up sampling was done 6 weeks after the beginning of the therapy and the findings were analyzed both on the Papanicolaou classification and the Bethesda system for cervical cytology 2001 [12]. The study was conducted during 2008 at Gyencology and Obstetrics Clinic "Narodni front" Belgrade. Study was approved by Institution Review Board of "Narodni front" Clinic, Belgrade.

The terminology for squamous epithelial lesions includes: atypical squamous cells of undetermined significance (ASCUS), squamous intraepithelial lesion (SIL), which encompasses the spectrum of squamous cell carcinoma precursors, divided into low-grade SIL (LSIL) (HPV-associated cellular changes and CIN1) and high-grade SIL (HSIL) (CIN2 and CIN3) [13].

Data obtained this study were analized by chi-square test, Fisher's exact test and Student *t*-test. The statistical program used was SAS® (ver 9.1).

Results

The parameters which were followed and analyzed in both groups were: age of examinees (presented as mean ± standard deviations), cytological findings in both examined groups and analysis of cytological findings prior and after therapy (the number of desquamous cells, the presence of cells of transformation zone, the level of atipicity of desquamous cells), as well as the presence of lactobacilli and pathogenic microorganisms (bacteria, fungi, protosoas) and white blood cells.

Mean age of examinees in the study group was 31 ± 8.12 years and in the control one 35 ± 9.77 years. The difference between groups regarding age was statistically significant ($p < 0.01$).

Cytology findings before and after therapy are presented in table 1.

Before the treatment there were no significant differences between the study and the control group, but after the treatment there were significant differences between the study and the control group in cytology findings ($p < 0.05$). The results presented in Table 1 showed that number of patients in the study group with signs of cervical inflammation and ASCUS was significantly higher before administration of probiotic preparation.

The results from the Table 2 showed that there were no significant differences between the study and the control group in discharge and composition of vaginal microflora before the treatmant.

However, there were significant differences between the study and the control group after the treatment indicating more efficient therapeutic response in the group treated with probiotic preparation. When compared results of the study group before and after the treatment, there were significant improvement in all indicators of the infection after the treatment with probiotics.

Representative microphotographs are presented in Figure 1.

Table 1

Cytology findings before and after therapy

Bethesda clasiffication	Papanicolaou test (PA)	Number of patients			
		Study group (n = 125)		Control group (n = 125)	
		before therapy	after therapy	before therapy	after therapy
Normal finding	PA I	79	109	90	96
Inflammation	PA II	19	4	19	15
ASCUS	PA IIIa	15	2	7	6
LSIL	PAIIIb-IV	5	4	6	5
HSIL		7	6	3	3

ASCUS - atypical squamous cells of undetermined significance; LSIL- low-grade squamous intraepithelial lesion; HSIL – high-grade squamous intraepithelial lesion

Table 2

The presence of vaginal infection indicators

Indicators of infection	Number of patients			
	Study group (n = 125)		Control group (n = 125)	
	before therapy	after therapy	before therapy	after therapy
Discharge	125	33	125	48
Lactobacilli	16	85	20	46
Bacteria	24	4	23	7
Fungi	35	9	37	27
Mixed flora	42	26	38	44
Trichomonas	8	1	7	1

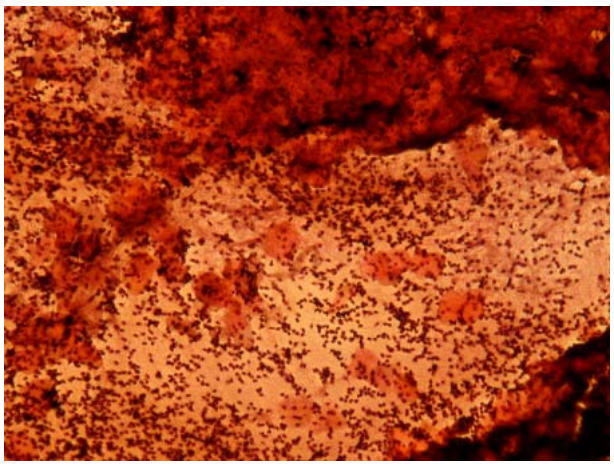

Fig. 1a – Inflammation – coilocytosis

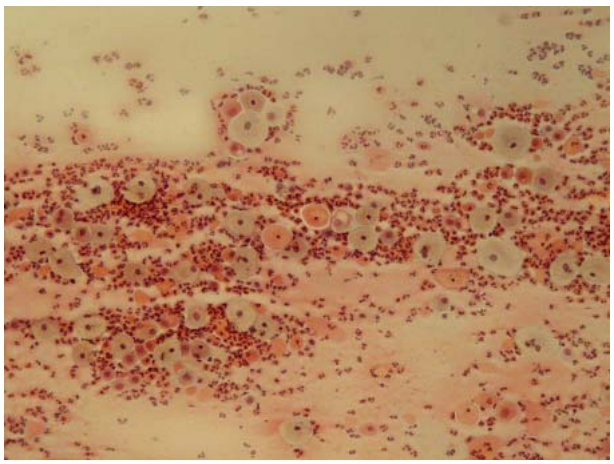

Fig. 1b – Inconclusive finding – inflammation

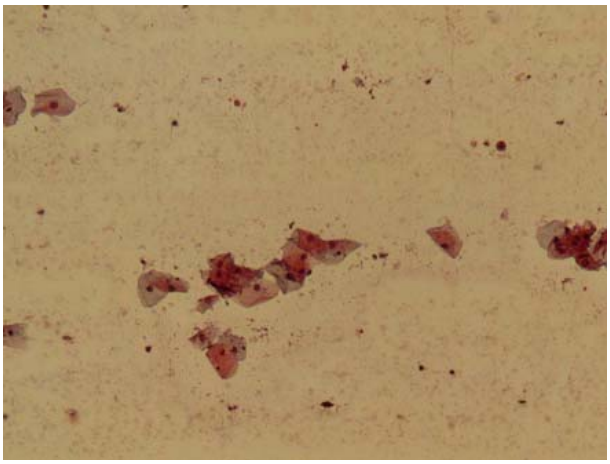

Fig. 1c – Inconclusive finding – red blood cell aggregate

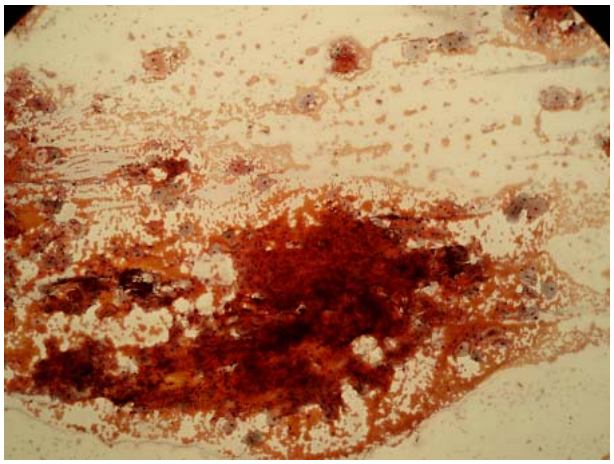

Fig. 1c – Cytological finding after the therapy

Discussion

Investigations of probiotic efficacy have shown benefits in reducing the recurrence of urogenital infections in women, while promising studies in cancer and allergies require further research for particular strains and better-designed trials [14]. It was found in *in vitro* studies that probiotics can inhibit the tumor growth and stop the growth of bacteria which play an important role in genesis of carinogens. It was shown that some products of probiotic bacteria like lactic acid has anticancerous effect by decreasing the activity of enzime beta-glukuronidase. All of these mentioned previously, can lead to conclusion that probiotics play an important role in prevention of colon cancer [15].

In the gastrointestinal and urogenital tract, fungal infections are very common [16]. The most common cause of fungal infections is *Candida albicans* (90–95%). There fungi are naturally located at intestinal mucosa, the skin, mouth and vagina, but in very small quantities. Multiplication of *Candida albicans* can be provoked by various factors such as antibiotics, stress, immune response changes, chemotherapy, hormonal contraception etc. Distruction of normal flora by the factors mentioned above provides that, candida can start grow uncontrolled and can colonize all of the gut, vagina, and so on. Not only yeast can grow uncontrolled. That can also be true for bacteria, especially *E. coli*. Prevention of all of these effects cannot be done only by diet changes itself. Therefore, it is recommended to take dietary suplements such are probiotics. Probiotics are very important factors in various infections prevention [17, 18]. Due to hormone level changes before menstrual bleeding and during pregnancy, the growth of yeast can be enhanced due to vaginal pH changes. Additionaly, during pregnancy, elevated level of oestrogen increases the blood glucose level which also leads to enhanced growth of vaginal candida.

Lactogyn® capsules contain two patented and for human use approved probiotic strains – *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 which are proved to be useful in prevention of bacterial vaginosis and candidiasis [19, 20]. Probiotics strains, enable maintenance of normal vaginal flora. It is thought that the mechanisms of lactobacilli action (supplemented orally) include: modulation of host immunity, reduction in pathogen ascension from the rectum, and interference with colonization and survival of pathogens [4]. It is suggested that combination of probiotic bacteria present in Lactogyn® should be taken during antibiotics therapy with the purpose of candidiasis prevention. However, it should be noted that probiotics (and Lactogyn®, as well), should be taken even without having symptoms and signs of the disease.

Not only for the prevention of yeast infection and support to antibiotics therapy, as seen in the study, Lactogyn® can enable faster, easier and more reliable cytological diagnosis. It, furthermore, can decrease the number of cytological analysis per woman, percent of false negative and false positive findings. The final result should be the decrease of presence of unsatisfactory and/or borderline satisfactory cytological findings. One recently published Italian study was focused on efficacy of the use of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 adminstred orally in the treatmen and prevention of vaginoses and bacterial vaginitis relapses. It was found out that these two probiotic strains, taken orally following antibiotic therapy, were much helpful in vaginosis and bacterial vaginitis treatment and in relapse prevention by re-establishing the vaginal ecosystem remarkably [21].

All of these mentioned above may lead to more efficient diagnosis and treatment which will directly influence the health of women with the reduction of costs in health system. Cervical carcinoma prevention and financial savings, should be the result of organized screening.

Conclusion

The use of the combination of probiotic bacteria *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 concomitantly with specific anti-infective therapy enables: more reliable cytological diagnostics, reduction of the percentage of false positive and false negative findings on cervical malignancy and reduction of unsatisfactory and/or borderline cytological findings. Also, it normalizes vaginal microflora in higher number of patients with vaginal infection comapred with specific anti-infective therapy, only.

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