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 examine 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, also, 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 6 weeks after beginning of the therapy a cervical smear cytological test (the Papnicolaou 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 compared 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 inhibisu stvaranje toksičnih supstanci i rast manje poželjnih vrsta boriće se za prostor i hranu. Lactogyn® je prvi oralni probiotik registrovan u Srbiji za zaštitu zdravlja vaginalne flore. Ovaj preparat sadrži dve dobro poznate probiotiske 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 sa dobro poznatih testova koji se koriste u dijagnostici maligniteta cerviksa uključujući detekciju atipičnih celija, detekciju lažno pozitivnih i lažno negativnih nalaza, kao i uticaj na vaginalnu mikrofloru. Metode. U studiji bilo je uključeno 250 žena sa znacima vaginalne infekcije. Studijsku grupu činilo je 125 žena kod kojih je započeta primena specifične antiinfektivne terapije i probiotikog preparata (Lactogyn® kapsule, 1 kapsula dnevno), a kontrolnu grupu, takođe, 125 žena koje su bile na odgovarajućoj antiinfektivnoj terapiji, ali bez dodatog probiotikog 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 infekcijom i atipičnim skvamoznim celijama neodređene značajnosti (ASCUS) bio je značajno veći pre upotrebe probiotika. Broj lactobacila bio je značajno
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.

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 been 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.

Lactogyn® is the first oral probiotic for restitution of vaginal flora balance and therefore may help, in a natural way, to establish and maintain urogenital health in women.

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 frequent due to the disturbance of vaginal flora. The supplementation 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 microscropy 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 antinfective 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.

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.
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. The study was conducted during 2008 at Gynecology 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).

Data obtained this study were analyzed 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, protozoas) 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.

Table 1

<table>
<thead>
<tr>
<th>Bethesda classification</th>
<th>Papanicolaou test (PA)</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before therapy</td>
<td>after therapy</td>
</tr>
<tr>
<td>Normal finding</td>
<td>PA I</td>
<td>79</td>
</tr>
<tr>
<td>Inflammation</td>
<td>PA II</td>
<td>19</td>
</tr>
<tr>
<td>ASCUS</td>
<td>PA IIIa</td>
<td>15</td>
</tr>
<tr>
<td>LSIL</td>
<td>PA IIIb-IV</td>
<td>5</td>
</tr>
<tr>
<td>HSIL</td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Indicators of infection</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group (n = 125)</td>
</tr>
<tr>
<td></td>
<td>before therapy</td>
</tr>
<tr>
<td>Discharge</td>
<td>125</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>16</td>
</tr>
<tr>
<td>Bacteria</td>
<td>24</td>
</tr>
<tr>
<td>Fungi</td>
<td>35</td>
</tr>
<tr>
<td>Mixed flora</td>
<td>42</td>
</tr>
<tr>
<td>Trichomonas</td>
<td>8</td>
</tr>
</tbody>
</table>

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. 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 carcinogens. It was shown that some products of probiotic bacteria like lactic acid has anticancerous effect by decreasing the activity of enzyme beta-glukuronidase. All of these mentioned previously, can lead to conclusion that probiotics play an important role in prevention of colon cancer.

In the gastrointestinal and urogenital tract, fungal infections are very common. 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. Distraction 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 but also bacteria, especially E. coli. Prevention of all 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. Due to hormone level changes before menstrual bleeding and during pregnancy, the growth of yeast can be enhanced due to vaginal pH changes. Additionally, 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. 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. 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 administered orally in the treatment 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 compared with specific anti-infective therapy, only.

**REFERENCES**


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