https://doi.org/10.31925/farmacia.2022.3.22 **ORIGINAL ARTICLE**

THE ROLE OF INTRAVAGINAL PREBIOTICS IN CONTROLLING THE EVOLUTION OF UNCOMPLICATED BACTERIAL AND FUNGAL VAGINAL INFECTIONS

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Abstract

Healthy vaginal flora is populated mainly by lactobacilli with an important role in protecting the host from urogenital infections. Conventional antimicrobial treatments have the risk of damaging healthy vaginal flora, which subsequently translates into significant recurrence rates. The aim of the study was to investigate if vaginal administration of α-glyco-oligosaccharides prebiotics, as specific nutritional support for lactobacilli, along with xylitol and hyaluronic acid could be considered as single treatment of uncomplicated vaginal infections (vaginitis or vaginosis) compared with classical antimicrobial preparations. The open-label, randomized, comparative study included 164 women between 18 and 66 years of age diagnosed with uncomplicated vaginal fungal or bacterial infections. Patients were randomized into 2 treatment groups: a prebiotic complex of α-glyco-oligosaccharides, hyaluronic acid, xylitol, herbal extracts, vitamin C and E for vaginal administration - group A, classic antimicrobial treatment with antibiotic and/or antifungal - group B. Patients have been evaluated before and 10 days after the start of treatment for symptomatic parameters (pruritus, burning, dyspareunia, dysuria, oedema, erythema, appearance and odour of vaginal secretions), microbiologic and microscopic analysis of vaginal secretions to identify pathogens, to assess the intensity of the inflammatory reaction (by polymorphonuclear cell density), and the density of the lactobacilli flora. The results showed that the vaginal administration of the prebiotic complex, xylitol and hyaluronic acid effectively controls the symptoms and pathogenic microbial load and, in unlike to the classic antibiotic and/or antifungal treatment, restores the healthy flora of lactobacilli and thus prevents the opportunistic development of *Candida*.

Rezumat

Flora vaginală sănătoasă este populată în special de lactobacili care joacă un important în protecția gazdei față de infecțiile urogenitale. Tratamentele antimicrobiene clasice prezintă riscul afectării florei vaginale sănătoase ceea ce se transpune ulterior în rate de recidivă importante. Scopul studiului a fost acela de a investiga daca administrarea vaginală de prebiotice din grupul α-glico-oligozaharide, ca suport nutritiv specific pentru lactobacili, împreuna cu xilitol si acid hialuronic poate reprezenta un tratament de sine stătător împotriva infecțiilor vaginale necomplicate comparativ cu tratamentul cu preparate antimicrobiene clasice. Studiul deschis, randomizat și comparativ a inclus 164 de femei cu vârste între 18 și 66 de ani, diagnosticate cu infecții vaginale fungice sau bacteriene (vaginite sau vaginoze) necomplicate. Pacientele au fost distribuite randomizat în 2 grupuri de tratament: un complex prebiotic de α-glico-oligozaharide, acid hialuronic, xilitol, extracte vegetale, vitamina C si E pentru administrare vaginală - grupul A, tratament medicamentos clasic cu antibiotic și/sau antifungic - grupul B. Pacientele au fost evaluate înainte și după 10 zile de la începerea tratamentului pentru parametri simptomatici (prurit, senzație de arsură, dispareunie, disurie, edem, eritem, aspectul și mirosul secrețiilor vaginale), analiza microbiologică și analiza microscopică a secrețiilor vaginale pentru identificarea patogenilor, evaluarea intensității reacției inflamatorii (prin densitatea celulelor polimorfonucleate), evaluarea densității florei de lactobacili. Rezultatele au arătat că prin administrarea vaginală a complexului de prebiotice se controlează eficient simptomatologia și încărcarea microbiană patogenă și, în plus față de tratamentul clasic cu antibiotic și/sau antifungic, se reface flora sănătoasă de lactobacili și astfel împiedică dezvoltarea oportunistă de *Candida.*

Keywords: oligosaccharides, xylitol, hyaluronic acid, vaginitis, vaginosis, lactobacilli

Introduction

The vaginal healthy human flora is dominated by lactobacilli, which play an important role in protecting the host from urogenital infections by inhibiting binding of other bacteria to epithelial cells and producing lactic acid that kills or inhibits the growth of many other bacteria [1]. Microbial balance between the dominant flora of lactobacilli and other microorganisms can be upset and this alteration of the ecosystem can lead to vaginal dysbiosis and infections with various adverse health outcomes such as bacterial vaginosis and aerobic vaginitis [2]. The imbalance can range from a low to a totally missing lactobacilli population. The cause is often the treatment of the urovaginal infection itself with antibacterial substances to which the most prevalent strains of lactobacilli are also sensitive [3]. Thus, the recurrence rate after treatments with antibacterial

substances is high and rise up to 50% within 6 months [4]. That is why in recent years there has been a major concern for the identification of therapeutic solutions for the prevention and treatment of vaginal infections that exclude the use of antibiotics and antifungals in order to preserve the healthy vaginal flora. One of the identified solutions is local application of oligosaccharides as prebiotics, specific nutrients that stimulate the growth of lactobacilli to the detriment of pathogens [5, 6]. Another one is local use of some short sugar alcohols (e.g. xylitol) in low concentrations (1 - 3%) that prevent the development of pathogens without interfering with lactobacilli vitality [7, 8]. But they are mainly studied as preventive treatment for rebalancing the vaginal flora in patients treated for vaginal infections with antibiotics and antifungals prior to prebiotics [9], or with prebiotics along with classical antimicrobial preparations [10]. The aim of the study was to investigate if vaginal administration of α-glyco-oligosaccharides prebiotics, as specific nutritional support for lactobacilli, in combination with xylitol and hyaluronic acid could be considered as single treatment for uncomplicated vaginal infections (vaginitis or vaginosis) compared with classical antimicrobial preparations.

Materials and Methods

Study population

The study was conducted in Obstetrics and Gynaecology department of Emergency Clinical Hospital Oradea, Romania, between 2018 and 2019 and was approved by Hospital's Ethical Committee. The open, randomised, comparative study included 164 women between 18 and 66 years old diagnosed with bacterial and/or fungal vaginal infections (vaginitis or vaginosis). The patients were randomly distributed in two treatment groups: Group A received for 10 days vaginal capsules and vaginal douche, that combine a prebiotic bio-complex of α-glyco-oligosaccharides, hyaluronic acid, xylitol and herbal extracts, vitamin C and vitamin E; Group B received a common recommendation for antibiotics (oral azithromycin or ciprofloxacin and local metronidazole) and/or local antifungal treatment (clotrimazole, itraconazole, isoconazole or nystatin).

The patients were included only with a signed informed consent and using the following inclusion criteria: patient over 18 years, of reproductive age or in menopause, pregnant or nursing, sexually active or not.

The patient for whom no informed consent was obtained, the patients with known history of hypersensitivity to any of the ingredients of the investigated products, immunocompromised patients (AIDS, malignancy, under chemotherapy/radiotherapy or immunosuppressive treatment) or patients with severe disease that need a more complex therapy than just antibiotic and/or antifungal treatment were excluded.

Evaluation criteria and outcomes

At the first visit the patient was evaluated for symptomatology and samples were taken for microbiological and microscopical analysis of vaginal discharge. The symptomatology was assessed using the following parameters: itching, burning, dyspareunia, dysuria, oedema, erythema, appearance and odour of the discharges using an analogic 4 points scale (0 - 3). Microbiological analysis identified the pathogen responsible for infection by culturing the pathogens on selective culture media and growing environment and by Gram staining features.

The microscopical analysis evaluated the polymorphonuclear cells (PMN) population density as a marker of inflammatory reaction intensity (severe: if PMN/epithelial cells ratio *per* field is > 1, moderate: if PMN/epithelial cells ratio *per* field is < 1, absent or neglectable [22]), the lactobacilli population density (normal: predominantly lactobacilli morphotypes, with very few coccoid bacteria present, reduced: diminished lactobacilli flora, which is mixed with other bacteria, absent [21]), and the presence/absence of *Trichomonas vaginalis*.

The same parameters were also evaluated at the end of each treatment and the adverse effects were reported as well.

Statistical analysis

Statistical significance of the results was checked comparing the initial and final results within each group and between groups using ANOVA, z-test two samples for mean and for a confidence interval of 95%.

Results and Discussion

Our study demonstrated that prebiotic combination of a bio-complex of α-glyco-oligosaccharides, xylitol, hyaluronic acid, and anti-inflammatory herbal extracts controls efficiently the symptomatology from uncomplicated vaginal infections (Table I).

Table I

Values for symptomatology parameters in each group (mean ± standard deviation, p value)

	Itching	Burning	Dyspareunia	Dysuria	Oedema	Erythema	Appearance	Odour
Group A (n = 104)								
Baseline	1.778 ± 1.09	0.375 ± 0.73	0.182± 0.51	0.288 ± 0.64	0.278 ± 0.68	0.240 ± 0.64	1.770 ± 1.13	0.125 ± 0.51
End of treatment	0.105 ± 0.39 p < 0.05	0 p < 0.05	0.019 ± 0.19 p < 0.05	0 p < 0.05	0 p < 0.05	0 p < 0.05	0.096 ± 0.38 p < 0.05	0 p < 0.05
Group B (n = 60)								
Baseline	1.283 ± 1.16	1.466 ± 1.32	0.433 ± 0.76	0.383 ± 0.76	0.266 ± 0.68	0.400 ± 0.82	1.316 ± 1.15	1.45 ± 1.44
End of treatment	0.783 ± 0.76 p < 0.05	0.033 ± 0.18 p < 0.05	0 p < 0.05	0 p < 0.05	0 p < 0.05	0.0166 ± 0.12 p < 0.05	0.8 ± 0.77 p = 0.001	0 p < 0.05

Table II

Microbiological analysis results in each group (n and % positive cultures)

	E. coli	*S. aureus*	*Enterococcus*	*Proteus*	*Gardnerella*	*Chlamydia*	*Enterobacter.*	*Klebsiella*	*C. albicans*	*Trichomonas*
Group A (n = 104)										
Baseline	17 16.34%	1 0.96%	2 1.92%	2 1.92%	5 4.80%	-	1 0.96%	-	80 76.92%	2 1.92%
End of treatment	0	0	0	0	0	-	0	-	3 2.88%	0
Group B (n = 60)										
Baseline	6 10%	1 1.66%	-	-	13 21.66%	8 13.33%	-	1 1.66%	33 55.00%	29 48.33%
End of treatment	0	0	-	-	0	0	-	0	44 73.33%	0

The antibiotic and/or antifungal common treatment had also a good response in controlling the symptomatology except for itching and the appearance of the vaginal discharge that still persists at the end of the treatment in a significantly higher degree than group A.

Microbiologic analysis reveals a pertinent and objective explanation for the subjective symptomatology results, namely the increased the amount of positive cultures of *C. albicans* in group B, a higher amount than in baseline results for this group while most of the pathogen cultures identified at baseline were negative at the end of the treatment in both groups (Table II). These results are not surprising since there are solid reports supporting the antibiotics role in dysbiosis of healthy flora in many body areas [13, 14, 15].

Microscopic analysis completes the microbiological picture for *Trichomonas* presence, confirming the efficacy of the treatment with prebiotic bio-complex of α-glyco-oligosaccharides, xylitol and hyaluronic acid (group A) with good results. However, it must be highlighted that group B included significantly more patients positive for *Trichomonas* (Table III).

Table III

Microscopical analysis results in each group (n and % of patients)

	Inflammatory reaction (PMN density)			Lactobacilli flora			*Trichomonas*	
	Severe	Moderate	Absent	Normal	Moderate	Absent	Present	Absent
Group A (n = 104)								
Baseline	65 62.5%	38 36.53%	1 0.96%	2 1.92%	10 9.61%	92 88.46%	2 1.92%	102 98.07%
End of treatment	1 0.96%	3 2.88%	100 96.15%	73 70.19%	31 29.80%	0	0	104 100%
Group B (n = 60)								
Baseline	45 75.00%	15 25.00%	0	0	8 13.33%	52 86.66%	30 50.00%	30 50.00%
End of treatment	0	26 43.33%	34 56.66%	1 1.66%	29 48.33%	30 50.00%	0	60 100%

Microscopic analysis also confirms objectively the symptomatology results, showing a persistent inflammatory reaction in antibiotic and/or antifungal common treatment group (group B) at the end of the treatment that is almost missing in group A (Figure 1).

The analysis results on lactobacilli population density remained after each treatment justifies the previous results and complete the picture of effectiveness as a whole (Figure 2).

The lactobacilli population density increased up to normal in group A and remained at an unsatisfactory and unprotecting level in group B. This explains the increased *Candida* presence along with the specific symptomatology (high score for itching, appearance of the discharge and PMN presence) in group B.

No patient reported any adverse effect throughout the treatment period.

From our knowledge it is the first report of the effectiveness of α-glyco-oligosaccharides and xylitol combination in eradication the pathogens with preservation of local healthy flora in vaginal administration; previous signalled reports have tested this combination to eradicate pathogen flora from skin [16]. Also most of the reports for vaginal use of prebiotics [9, 10] explore their efficacy using instruments that pointed out only their preventive action, such as Nugent score and vaginal smear pH that provide information only regarding lactobacilli population *versus* and some strains involved in bacterial vaginosis (*Gardnerella*). The present study uses effective clinical instruments of investigation that provide valuable evidence for the curative action of prebiotics, (e.g.

microbiological cultures, PMN density), not only preventive.

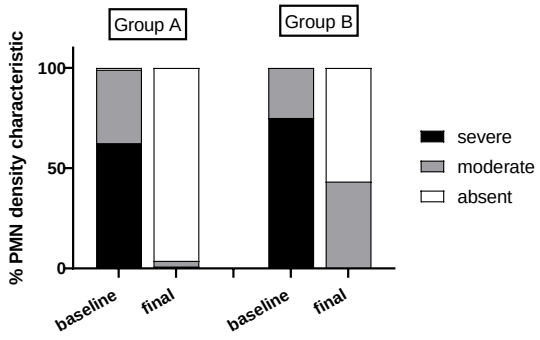

Figure 1.
Evolution in inflammatory reaction as PMN density (% patients)

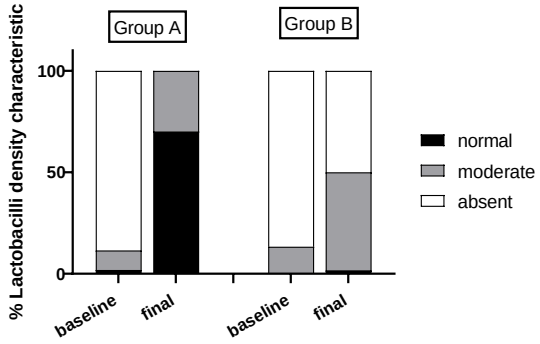

Figure 2.
Evolution in lactobacilli population density (%patients)

It also must be noted that literature is abundant in reports of local use of mainly one isolated and purified lactobacilli strain for the control of symptomatology and pathogen load in vaginal infections. But prebiotics combination that includes oligosaccharides have an important advantage: oligosaccharides are metabolised as specific nutrient support by a wide range of protective lactobacilli strains that includes not only *L. rhamnosus* and *L. reuterii*, but also *L. acidophilus, L. casei*, *L. plantarum* [17] and thus increases the chance of repopulation with a more diverse flora, closer to the one found in healthy vaginal environment [18]. Also, unlike probiotics, prebiotics are not affected by simultaneous treatment with antibiotics, if this type of treatment is deemed necessary.

Standard treatment for vaginal infection with antibiotic and/or antifungal, oral or local is associated with high cure rate one week after the treatment [19]. However, cure rate decreases with time and relapses are more and more frequent [4]. But this is not the only concern; frequent antibiotic treatments and also metronidazole exposure (the main therapeutic intervention for vaginosis with anaerobes [20]) is associated with *Candida albicans*, other fluconazole-resistant *Candida* isolates or *C. glabrata* infections [13]. This is the result of a reduction in vaginal protecting lactobacilli population of different causes among which the main cause remains the common antibiotic and/or antifungal treatment itself [14]. That is why it becomes very important to seek for alternative solutions that act therapeutically against pathogens and symptoms and restore, or at least do not harm, the protective vaginal lactobacilli barrier. In this area of concern, there have been previous reports on antimicrobial effectiveness of ascorbic acid, oligosaccharides and lactobacillus strains combination as a vaginal insert [21] and also on hyaluronic acid and vitamins A and E combination effectiveness in controlling vaginal inflammation symptoms as itching, burning and dyspareunia [22]. The present study joins and supports this increasing trend.

Conclusions

Vaginal administration of prebiotic bio-complex of α-glyco-oligosaccharides, combined with hyaluronic acid, xylitol, herbal extracts, vitamin C and vitamin E is effective in managing the symptomatology and pathogen load in uncomplicated vaginal infection as a single treatment in patients who have not been previously treated with a common antibiotic/ antifungal treatment. The certain benefit of the proposed treatment is the recovery and support for the healthy vaginal flora comparative with common antibiotic/antifungal treatment.

Conflict of interest

The authors declare no conflict of interest.

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