



Microbiological Assessment of Glycyrrhizic Acid Effectiveness in Bacterial Vaginosis – A Comparative Study

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Abstract

Background. Bacterial vaginosis (BV) is one of the most common female diseases, which is currently characterized by an increasing rate of clinical sign recurrence, the appearance of asymptomatic carriers, and atypical forms.

This study was aimed to evaluate the effectiveness of glycyrrhizic acid administration in the prevention of BV recurrence.

Materials and Methods. The study involved 88 women after BV treatment (metronidazole *per os* for seven days). Participants were randomly divided into two groups: the main group included 46 patients who were administered 0.1% glycyrrhizic acid intravaginally for three months after the main treatment; the control group comprised 42 women who received no anti-recurrence BV course. The effectiveness was assessed three and six months after the beginning of treatment and included gynecological examination and laboratory assessments (pH, amine test, microbiological investigation).

Results. After recommended BV treatment without anti-recurrence course, the recurrence of laboratory criteria for BV increased three months after the treatment, including the increase in vaginal pH to > 4.5 in all subjects, positive amine test in 35.7% of patients, the presence of *Gardnerella vaginalis* at a concentration of 10⁴-10⁵ CFU/ml in 31.0% of women, reduction in the frequency of Lactobacillus detection with worsening of the condition and the onset of clinical manifestations of the disease six months later. In the main group, after glycyrrhizic acid administration, laboratory criteria of BV recovery three months after the treatment, and clinical and laboratory markers of BV recovery six months after the treatment were lower as compared to the control group.

Conclusions. The anti-recurrence course of BV (vaginal administration of 0.1% glycyrrhizic acid) demonstrated a decrease in the frequency of complaints, clinical and laboratory markers, stabilization of the species composition of Lactobacillus, and a decrease in facultative anaerobe concentration in the vaginal microbiome as compared to group without anti-recurrence treatment.

Keywords

Bacterial Vaginosis; Vaginal Microflora; Facultative Anaerobic Bacteria; Lactobacilli; Glycyrrhizic Acid

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Introduction

Bacterial vaginosis (BV) is a condition caused by an overgrowth of normal vaginal flora on the background of a decrease in the number of normal lactobacilli producing hydrogen peroxide, with an overgrowth of anaerobic bacteria [1, 2]. Historically, BV was called Gardnerella vaginitis, as *Gardnerella vaginalis* was believed to be the main cause of this condition [3]. However, a new term, BV, emphasizes

that various microorganisms present in the vagina can grow in excess and cause a pathological condition [4]. The most common clinical manifestation of BV is an increased gray to white vaginal discharge with a foul odour. Women with BV are known to be at an increased risk of getting other sexually transmitted infections, while pregnant women with BV are at high risk of preterm delivery [5–7].

Studies conducted on BV showed an increase in the rate of clinical sign recurrences, the appearance of asymp-

omatic carriers and atypical forms [8]. A variety of approaches to cure BV, including the use of antibiotics, probiotics, or bacteriophage therapy, are continuously considered by clinicians globally. Antibiotics used for the treatment of BV include oral or intravaginal metronidazole and clindamycin [9]. However, there has been reported a recurrence rate of 76% with onset within six months after treatment, probably due to antibiotic resistant pathogenic bacteria and their biofilms [10, 11].

Probiotic therapy, administering lactobacilli for the recolonization of normal vaginal microorganisms, is considered as the second-line treatment of BV [12]. This therapy, however, showed positive results only in case of long-term use of probiotics (1-3 months) as compared to short-term treatment (< 1 month) [13]. According to Wu *et al.*, a lot of factors such as resident vaginal bacteria, glycogen and lactic acid concentration, sexual intercourse, hormonal changes, and local immune response status can influence *Lactobacillus* colonization [14]. Therefore, improving existing treatment approaches with evidence-based and cost-effective options remains relevant and discovers new perspectives in clinical practice.

Glycyrrhizic acid (GA) is the main active component of licorice (*Glycyrrhiza glabra*) root, that is composed of a hydrophilic part, two molecules of glucuronic acid, and a hydrophobic part, glycyrrhetic acid. GA undergoes acid hydrolysis and releases 18 β - and 18 α -glycyrrhetic acids, which determine the high beneficial pharmacological activity of licorice [15].

The antiviral, anti-inflammatory, antitumor, and antimicrobial effects of GA have been reported. The anti-inflammatory property of GA was demonstrated in the treatment of radiation-induced skin injury [16]; the anti-allergic effect, reducing erythema, oedema, and itching symptoms, was shown in a double-blind clinical trial in the treatment of atopic dermatitis [17]. GA induces the production of host's own interferons. The anti-inflammatory activity of activated GA is combined with a stimulating effect on the humoral and cellular factors of immunity; it significantly inhibits kinin release and prostaglandin synthesis via connective tissue cells at the site of inflammation. 18 β -glycyrrhetic acid, as an active component of GA, has a regenerating effect resulting in proper repair of the skin and mucous membranes [18].

The therapeutic spectrum of GA effects includes antimicrobial activity through the inhibition of bacterial infection by reducing gene expression, inhibiting bacterial growth, and reducing the production of microbial toxins [19]. Thus, the alcoholic extract of *G. glabra* roots showed significant antibacterial activity, with a formation of growth inhibition zones against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Bacillus cereus* [20].

GA has demonstrated efficacy in the treatment of intestinal candidiasis [21], fungal vulvovaginitis [22, 23], vaginitis [24], vestibulodynia [25]. This study was aimed to evaluate the effectiveness of GA in the prevention of BV recurrence.

Materials and Methods

Study Design

This single-center comparative study was carried out at the City Clinical Perinatal Center, Ivano-Frankivsk, Ukraine, between April 2021 and May 2022.

Study Participants and Eligibility Criteria

A total of 88 women with BV were enrolled in the study. Inclusion criteria were:

- BV according to Amsel's criteria [9],
- participants aged between 18 to 45 years,
- no severe somatic pathology,
- no acute infectious process,
- no antibiotic exposure during the last six months,
- using condoms during every act of sexual intercourse,
- consent to participate in the study.

Exclusion criteria were women aged under 18 and over 45 years with severe somatic and endocrine diseases, sexually transmitted infections, acute pelvic inflammatory disease.

BV Treatment

All patients received a course of antibacterial therapy for BV - metronidazole 500 mg orally twice a day for seven days, according to the recommendations [9], with control of clinical and laboratory results of recovery at the time of their enrollment in the study.

Study Groups and Anti-Recurrence Treatment

After antibacterial therapy for BV, women were divided into two groups: the main group included 46 subjects who were administered 0.1% GA intravaginally by 1-2 pressures through a special tube for five days following the main treatment, with repeated 5-day anti-recurrence courses immediately after menstruation for three months; women of the control group (42 patients) received no anti-recurrence treatment.

Assessment

The effectiveness of the proposed course was assessed based on the clinical and laboratory data three and six months after the end of the main treatment.

Clinical and laboratory assessments included gynecological examination, determination of vaginal pH and amine test (AT) (reaction with a 10% solution of potassium hydroxide), microscopy of Gram-stained vaginal smears, bacteriological cultures on nutrient media for facultative anaerobes (FAs), quantitative analysis of the vaginal microflora.

Gardnerella vaginalis was isolated in an atmosphere enriched with carbon dioxide on anaerobic blood agar with the addition of gentamicin sulfate, nalidixic acid, and amphotericin in adequate proportions. *Staphylococcus* and *Streptococcus* strains were identified by slide tests (Difco, USA; BioMerieux, France); cultures of lactobacilli were identified by the "System of Indicator Papers for Identification of LB". For the quantitative determination of FAs, the method of sectoral crops was used. The concentration of 10⁴ CFU/ml was considered a barrier level in the quantitative determination of microorganism pathogenicity.

Statistical Analysis

The data were analyzed using Statistica 12.0 (StatSoft Inc., USA). The descriptive data are presented as Mean \pm Standard Deviation (Mean \pm SD) for continuous variables (pH) and percentages for discrete data. The Chi-square test was applied to compare percentages; non-parametric tests (the Mann-Whitney U test and the Wilcoxon signed-rank test) were used to identify changes in pH-values. The differences between the groups were considered statistically significant at $p < 0.05$.

Results

The mean age of patients in the main group was 28.4 ± 4.5 years, in the control group – 27.0 ± 5.3 years ($p > 0.05$). The period from BV symptom onset to the beginning of treatment included 6.0 ± 1.3 days in the main group and 6.3 ± 1.5 days in the control group ($p > 0.05$). After the three-month follow-up, the recurrence of clinical symptoms did not differ significantly between the groups ($p > 0.05$) (Table 1).

However, six months after treatment, in the main group, there was a significantly lower incidence of recurrent vaginal discharge (5.1-fold), bad-smelling discharge (4.5-fold), itching (4.3-fold), dyspareunia (3.8-fold) as compared to the control group.

After BV treatment, pH values did not differ between the groups ($p > 0.05$). In the main group, three and six

months after the treatment, pH increased slightly, but not significantly. However, in the control group, its value gradually increased by 1.29 and 1.38 times, respectively, as compared to the baseline and was significantly higher than respective values of the main group.

In the control group, positive AT was observed in 35.7% and 95.2% of women three and six months after the treatment, respectively, while in the main group, positive AT was found only in 3 (6.5%) women six months after the treatment.

There were significant differences in the qualitative characteristics of the vaginal microbiome. After BV treatment, women of both groups were diagnosed with vaginal normocenosis with Lactobacillus dominance, represented by the main three cultures – *L. acidophilus*, *L. fermentum*, and *L. plantarum* at a concentration of 10^7 - 10^8 CFU/ml (Table 2). However, only 54 out of 88 patients (61.4%) had the associations of two or more species of lactobacilli, the rest had a monoculture; 86.4% of isolated lactobacilli had the ability to produce hydrogen peroxide, which indicated their rather high protective capacity.

Three months after the treatment, lactobacilli dominated in the vaginal microbiome in women of both groups. The most common species were *L. acidophilus*, *L. fermentum*, and *L. plantarum* at concentrations of 10^6 - 10^8 CFU/ml; their detection frequency and concentrations did not differ between the groups ($p > 0.05$).

Six months after the treatment, the composition of

Table 1. Complaints of women under study three and six months after the treatment (abs., %).

Complaints	Group		p*
	Main (n=46)	Control (n=42)	
Significant vaginal discharge:			
– three months	1 (2.2)	4 (9.5)	0.3
– six months	6 (13.0)	28 (66.7)	<0.001
Bad-smelling discharge:			
– three months	2 (4.3)	3 (7.1)	0.91
– six months	5 (10.9)	21 (50.0)	<0.001
Vaginal itching:			
– three months	1 (2.2)	4 (9.5)	0.3
– six months	4 (8.7)	16 (38.1)	0.002
Dyspareunia:			
– three months	0 (0.0)	0 (0.0)	-
– six months	4 (8.7)	14 (33.3)	0.009
pH:			
– baseline	3.71 ± 0.31	3.73 ± 0.32	
– three months	3.79 ± 0.36	$4.81 \pm 0.36^\#$	<0.05
– six months	4.05 ± 0.42	$5.16 \pm 0.36^\#$	<0.05
Amine test:			
– three months:			
• negative	46 (100.0)	27 (64.4)	<0.001
• positive	0 (0.0)	15 (35.7)	<0.001
– six months:			
• negative	43 (93.5)	2 (4.8)	<0.001
• positive	3 (6.5)	40 (95.2)	<0.001

Notes: * – comparison of the main and control groups; # – as compared to the baseline level indicator ($p < 0.05$).

Table 2. Species composition and frequency of detecting lactobacilli in vaginal secretions of examined subjects.

Group	Lactobacilli				
	<i>L. acidophilus</i>	<i>L. fermentum</i>	<i>L. plantarum</i>	<i>L. coryneformis</i>	<i>L. paracasei</i>
After a course of antibacterial therapy					
Main, % (n=46)	100.0	65.2	54.3	-	-
Control, % (n=42)	100.0	66.7	57.1	-	-
Three months after the treatment					
Main, % (n=46)	73.9	52.2	41.3	4.3	4.3
Control, % (n=42)	64.3	47.6	40.5	2.4	2.4
Six months after the treatment					
Main, % (n=46)	56.5	47.8	41.3	6.5	2.2
Control, % (n=42)	31.0 [#]	23.8 ^{#*}	16.7 ^{#*}	2.4	2.4

Notes: # – p<0.05 as compared to the indicator in the group three months after the treatment; * – p<0.05 as compared to the indicator of the main group.

the vaginal microbiota in the control group changed: there was a 2-2.8-fold decrease in Lactobacillus detection (p < 0.05). In all cases, lactobacilli were isolated as a monoculture; 11.9% of lactobacilli produced hydrogen peroxide, which indicated their low protective capacity.

In all women of the main group, six months after the treatment, the frequency of detecting lactobacilli decreased, but not significantly; however, it was significantly higher (1.8-2.5-fold) as compared to the control group. In the main group, 89.1% of isolated lactobacilli had the ability to produce hydrogen peroxide, which indicated their high protective capacity.

After the main course of BV therapy, FAs were always found in monoculture at a concentration < 10³ CFU/ml. *E. coli* and *Staphylococcus epidermidis* were isolated in 10 out of 88 (11.4%) women, *S. saprophyticus* and *Proteus mirabilis* were isolated in 3 (3.4%) women, *Corynebacterium spp.* were isolated in 2 (2.3%) women; *Gardnerella vaginalis* was detected in no participant.

Examining the composition of the vaginal microbiome in women under study within the observation periods revealed significant differences between the groups. Three months after the treatment, FAs were found in monoculture at a concentration less than 10³ CFU/ml in patients of the main group (Table 3). In the control group, the following microorganisms were significantly more common: *E. coli* – in 33.3% of cases, *Gardnerella vaginalis* – in

31.0% of women, *S. epidermidis* – in 35.7% of patients (p < 0.05). Six months after the treatment, in the main group, FAs were detected in monoculture, the frequency did not differ from that in the three-month period (p > 0.05), and the vaginal microbiome was characterised by vaginal normocenosis. In the control group, *E. coli* and *Gardnerella vaginalis* were found more common (p < 0.05) at higher concentrations as compared to the main group (p < 0.05). In 17 out of 42 (40.5%) women of the control group, FAs were found in an association of two and three microorganisms.

Discussion

The obtained results showed that six months after recommended antibacterial BV therapy, the frequency of recurrent disease complaints increased significantly. According to laboratory assessments in the control group, three months after the treatment, a 1.3-fold increase in vaginal pH level was found in all subjects; AT was positive in 35.7% of women; *Gardnerella vaginalis* at a pathological concentration of 10⁴-10⁵ CFU/ml was present in 31.0% of women, with a significant deterioration six months after the treatment (as compared to the main group), which indicated the laboratory-confirmed recurrence of BV before clinical manifestations. However, in the main group, six months after the treatment, the frequency of complaints

Table 3. Facultative anaerobes identified in the vaginal microbiome.

	After the treatment							
	Three months				Six months			
	Main Group		Control Group		Main Group		Control Group	
	n	CFU/ml	n	CFU/ml	n	CFU/ml	n	CFU/ml
<i>E. coli</i>	4	<10 ³	14 [*]	10 ³ -10 ⁴	8	10 ³	17 [*]	10 ⁴ -10 ⁵
<i>S. epidermidis</i>	7	<10 ³	15 [*]	10 ³ -10 ⁴	10	10 ³	15	10 ⁴ -10 ⁵
<i>Corynebacterium spp.</i>	3	<10 ³	3	<10 ³	5	10 ³	2	10 ⁴
<i>S. saprophyticus</i>	2	<10 ³	2	<10 ³	3	10 ³	1	10 ³
<i>Proteus mirabilis</i>	1	<10 ³	1	<10 ³	1	10 ³	1	10 ³
<i>Gardnerella vaginalis</i>	5	<10 ³	13 [*]	10 ⁴ -10 ⁵	1	10 ³	23 [*]	10 ⁶ -10 ⁷

Note: * – as compared to the main group (p<0.05).

(vaginal discharge, odor, itching, and dyspareunia) was significantly lower (by 3.8–5.1, $p < 0.01$) as compared to the control group.

The results of this study showed that in the absence of anti-recurrence BV course, already three months after the treatment, the regression of Lactobacillus pool was accompanied by increased composition and concentration of FA flora (preclinical manifestations of vaginal dysbiosis) and a high frequency of occurrence of clinical and laboratory criteria for BV six months after the treatment. In a study by Vodstrcil *et al.*, there was highlighted a low effect of the main antibacterial treatment due to limited understanding of the pathogenesis of BV recurrence, possible persistence and re-emergence of BV-associated bacteria or a BV-associated biofilm following antimicrobials and/or re-infection occurring from sexual partners [8]. Moreover, McKinnon *et al.* reported the presence of asymptomatic BV, a condition associated with cervicovaginal microbiota dominated by *Gardnerella vaginalis* or polymicrobial microbiota containing facultative and/or obligate anaerobes, no *Lactobacillus spp.*, but without any special complaints [26]. Our results confirmed an increase in the frequency of detecting *Gardnerella vaginalis* in subjects of the control group during the observation period (31.0–54.8%); however, it was accompanied by clinical and laboratory indicators of BV.

Rapid BV recurrence following standard antibiotic treatment has generated substantial interest in alternative treatment strategies and antibiotic adjuncts, including the use of Lactobacillus probiotics or live biotherapeutics, either alone or in combination with antibiotics. However, according to Wang *et al.*, not all probiotics reduce the recurrence rate of BV [27], and in a study by Happel *et al.*, insufficient effect of Lactobacillus probiotics has been shown [28].

The present study demonstrated the effectiveness of intravaginal administration of 0.1% GA in the complex anti-recurrence therapy of patients with BV. The results demonstrated that in the group of patients after prolonged preventive therapy, there was a statistically significant decrease in laboratory criteria for BV, clinical and laboratory markers of BV three and six months after the treatment, respectively. Other studies showed a high clinical and laboratory efficiency of using intimate hygiene products based on ammonium glycyrrhizinate and lactic acid, both in the complex therapy for patients with vaginal microbiome disorders and concomitant viral infections, and in monotherapy for restoring microbiocenosis disorders [22, 29, 30]. In a study by Pagano *et al.*, bioadhesive, vaginal emulgels loaded with the hybrids of 18 β -glycyrrhetic acid as the main bioactive component of licorice roots and rhizomes of *Glycyrrhiza glabra* species were developed for systemic and local application. They demonstrated mucoadhesive property to the vaginal mucosa, necessary to prolong the residence time at the site of application [18].

The effectiveness of GA in the prevention of BV clinical symptom recurrence, observed in our study, is likely due to the anti-allergic effect on immunoglobulin (Ig) E-mediated allergic response demonstrated by Han *et al.* [31]. A study by Dodangeh *et al.* showed that using *Glycyrrhiza*

glabra vaginal cream for treatment of fungal vulvovaginitis led to a decrease in vaginal discharge, itching, irritation and burning, painful urination and intercourse, vulvovaginal redness, and cheese-like discharge [22]. According to the authors, the normalization of the vaginal microbiome six months after BV therapy following recommended anti-recurrence treatment was potentially ensured by the antimicrobial effect of GA due to growth inhibition of gram-positive and gram-negative bacteria and the negative effect on their metabolism [20].

Limitations

The results of this study should be considered in light of some limitations. Firstly, it is a single-center comparative study limited by sample size; secondly, participant's eligibility criteria could potentially affect the study findings and patients with comorbidities were not included in the study; finally, the anti-recurrence course was based on GA administration only, and its effect with other medicines was not assessed in this study.

Conclusions

Vaginal administration of 0.1% GA in an anti-recurrence course following standard BV therapy resulted in a sustained reduction in clinical and laboratory markers, stabilization of Lactobacillus pool, and regression of FAs in the vaginal microbiome as compared to group without anti-recurrence treatment.

Ethical Statement

The design of the research was approved by the Ethics Committee of the Ivano-Frankivsk National Medical University. The study was conducted according to the WHO Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

Informed Consent

Informed consent was obtained from all the patients.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that no conflicts exist.

Financial Disclosure

The authors declared no financial support.

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