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Microbiocenosis of the Neovagina and Intestines in the Postoperative Period

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Abstract Today, the number of patients requiring colpopoiesis surgery is increasing significantly. In the postoperative period, much attention is paid to the problem of vaginal microflora and is an urgent topic that plays an important role in assessing a woman's reproductive health. There is a wide variety of data on the microflora of the artificial vagina, which varies depending on the stage of the rehabilitation period after surgery for colpopoiesis, described by a small number of authors, which does not allow standardizing these indicators. This article presents data on the microbial flora of the sigmoid neovagina.

Keywords Sigmoid neovagina, Colpopoiesis, Microbiology, Bacterial flora

1. Introduction

One of the most significant problems of modern health care around the world is the problem of reproductive and sexual health. Anomalies of the uterus and vagina are congenital defects that have become increasingly common in recent years. Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS) is a leading cause of vaginal and uterine aplasia with an incidence of 1 in 4500 female births. In recent decades, a number of scientific studies have been carried out all over the world to develop the most evidence-based treatment methods and identify risk factors for abnormal development of the genital organs in girls, such as uterine and vaginal defects (Negmadzhanov B.B., 2021; Kirpatovsky I.D., Ugryumova L. Yu, Uvarova E. V., 2017). Treatment of Rokitansky-Küstner syndrome is surgical and comes down to the creation of a neovagina [3,4,8,9]. To create a neovagina, sigmoid colpopoiesis is successfully used (Negmadjanov B.B. et al., 2020).

After sigmoidal colpopoiesis, pathogens of bacterial infections often develop and persist for a long time, causing colpitis, bacterial vaginosis, trophic changes in neovaginal tissue, which requires complex therapy, taking into account the microbial factor and the severity of the inflammatory process (Navruzov S. N., Navruzov B. S., Shaimardanov E.K., 2017). It is the saturation of the vaginal wall with lactobacilli in the postoperative period that makes it possible to change the qualitative composition of the microflora, thereby having a beneficial effect on the course of the postoperative period, affecting the outcomes of surgical treatment [2,5,6]. There are reports of high therapeutic and prophylactic effectiveness of probiotics in inflammatory processes of various localizations, including purulent-inflammatory complications after surgery (Andreeva I.V., 2015). The study of vaginal microbiocenosis after sigmaid colpopoiesis and the improvement of methods of clinical and laboratory diagnosis of this disease will contribute to the understanding of the mechanisms of the pathogenesis of inflammatory diseases, complicating its course and more effective tactics for managing patients. This pathology, while remaining stable and not threatening the patient’s life, causes severe physical and moral suffering due to the emergence of a characteristic “symptom complex”, in which developing complications play an important role, with the ensuing adverse consequences [1,7,8].

2. Purpose of the Study

To develop preventive measures to reduce the incidence of postoperative infectious and inflammatory complications.

3. Material and Research Methods

The study included 81 patients after undergoing sigmaid colpopoiesis, who were divided into 2 groups: the main group - 49 patients with complaints of heavy discharge from the genital tract and the control group - 32 women without clinical signs of bacterial vaginosis.

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4. Results of the Study

When studying the anamnesis of the women who took part, special attention was paid to such parameters as: age, social status and material and living conditions, education, characteristics of professional activity, occupational hazards; diseases suffered in different years of life, their course, outcome; the presence of diseases that are a manifestation of connective tissue failure (varicose veins, hernias of various locations, a history of frequent joint dislocations); the presence of diseases accompanied by increased intra-abdominal pressure (cough, constipation); complaints made by a woman upon admission to hospital, main symptoms.

The study of vaginal microbiocenosis was carried out using complex quantitative real-time PCR using Femoflor-16 reagents in a detection amplifier in 81 patients. The Femoflor-16 reagent kit is intended for the detection of DNA of opportunistic microorganisms, lactobacilli and human genomic DNA (as a control for taking biological material).

Table 1. Number of microorganisms detected in the negative control sample

Femoflor-16		
№	**Name of microorganism**	**Quantity in K-, Lg**
	Total bacterial mass	no more 3,5
1	*Lactobacillus spp.*	no more 2,5
2	*Сем. Enterobacteriaceae*	no more 2,5
3	*Streptococcus spp.*	no more 2,5
4	*Staphylococcus spp.*	no more 2,5
5	*Gardnerella vagmalis /Provotella bivia /Porhylomonas spp.*	no more 2,5
6	*Eubacterium spp.*	no more 2,5
7	*Sneathia spp. /Leptotrihia spp. / Fusobactenum spp.*	no more 2,5
8	*Megasphaera spp. /Veillonella spp. /Diahster spp.*	no more 2,5
9	*Lachnobacterium spp. /Clostridium spp.*	no more 2,5
10	*Mobiluncus spp. /Corynebactenum spp.*	no more 2,5
11	*Peptostreptococcus spp.*	no more 2,5
12	*Atopobium vaginae*	no more 2,5
13	*Mycoplasma (hominis +gemtalium)*	Absent
14	*Ureaplasma (urealyticum + parvum)*	Absent
15	*Candida spp.*	no more 3,0

Quantitative assessment of the urogenital biota was carried out in absolute and relative terms (Table 1). To quantify the normal flora and UPM, relative indicators were used, which were calculated as the difference in logarithms to base 10, using the formula: log10(-) = log10x - log10y. The relative indicator of normal flora was the difference in logarithms obtained for the total bacterial mass and normal flora. The test determines the total concentration of bacterial DNA - total bacterial mass (TBM) - and the concentration (absolute and relative) of the following types/genera of microorganisms: Lactobacillus, Enterobacteriaceae, Streptococcus, Staphylococcus, Gardnerella vaginalis/ Prevotella bivia/ Porphyromonas, Eubacterium, Sneathia/ Leptotrichia/ Fusobacterium, Megasphaera/ Veillonella/ Dialister, Lachnobacterium/ Clostridium, Corynebacterium/ Mobiluncus, Peptostreptococcus, Atopobium vaginae. The ratio of these bacteria determines the state of the vaginal microbiocenosis - normocenosis or dysbiosis. Dysbiosis, in turn, is assessed by the degree of severity (moderate or severe dysbiosis) and the predominance of aerobic or anaerobic conditionally pathogenic microflora (aerobic or anaerobic dysbiosis, respectively). A study of intestinal microflora was carried out on 81 patients in accordance with the methodological recommendations of N.M. Grachevoy et al., (1986) [Gracheva N.M., 1986; Znamensky V.A. et al., 1986; Raevsky K.K. et al, 1997; Dobrynin V.M. et al., 1999; Efimov B.A. et al., 2002, etc.;]. The state of intestinal microbiocenosis was assessed in all patients upon presentation. Determination of the species of bacteria was carried out using generally accepted methods and identification schemes (Table 2). Microbial colonization of the intestine was assessed by the frequency of isolation of symbionts and by intensity - CFU/g, the tenth logarithm of the average number of microorganisms (lg CFU) isolated from the test material. Assessment of dysbacterial abnormalities in the intestines according to the degree of dysbiosis was carried out according to the classification of Kuvaev I.B., Ladodo K.S. (1991) [Kuvaeva I.B., Ladodo K.S. Microecological and immune disorders in children. M.: Medicine; 1991; p.240].

According to the generally accepted classification, intestinal dysbiosis was assessed according to four degrees of severity (Table 3).

The biocenosis of the neovagina and intestines in women was studied using RT-PCR. As a result of the study, significant changes were established in the structure of the vaginal biocenosis among the examined groups of women.

Moderate anaerobic dysbiosis in general in women with neovagina in the main group was found 4.5 times more often than in the control group (14.28% versus 3.12%; $P < 0.05$). A pronounced degree of anaerobic dysbiosis was identified in 20 (40.82%) women. Its etiological structure was made up of pathogens associated with bacterial vaginosis: Gardnerella vaginalis, Atopobium vaginalis and Ureaplasma in various combinations. The most frequently detected were Gardnerella vaginalis (26) and Ureaplasma (urealyticum+parvum).

Indicators of bacterial vaginosis in women are mainly determined in the following quantities: Gardnerella vaginalis - in 37.03% (30/81) of cases, Ureaplasma spp. and fungi of the genus Candida in diagnostic titers - in 27.1% (22/81) and 9.8% of cases (8/81), Mobiluncus species - in 13.5% of cases (11/81), Atopobium vaginae - 7, %4 (6/81). In the control group: Gardnerella v. – 3.3%, Ureaplasma spp. – 3.3%, Atopobium v. – 3.3%. In 13 cases (16.04%), facultative anaerobic microorganisms Streptococcus spp., Staphylococcus spp and Enterobacteriaceae were identified, causing inflammatory processes in women, and it should be noted that in most cases in women of the main group.

Table 2. Species of Intestinal Bacteria being Studied

Microorganisms	Culture media	Cultivation conditions
Bifidobacteria	Blaurock medium modified by G.I. Goncharova	3 days at 37°C
Bacteroides	Selective Haenel agar, MPA slant	4-5 days in a microanaerostat
Spore-forming anaerobes (clostridia)	MRS-agap and LBS-agap	4-8 days at a temperature of 37°C
Enterococci	Wednesday Kalina, DIF-3	2-3 days at a temperature of 37°C
Staphylococcus	blood agar, yolk salt agar	2 days at 37°C
Yeast-like fungi	Sabouraud's medium with polymyxin	1 day at a temperature of 37°C, then 2-5 days at 22°C
Enterobacteriaceae (Escherichia, Klebsiella, Proteus and others), Pseudomonas aeruginosa	endo blood agar medium	1 day at 37°C

Table 3. Classification of Intestine Dysbiosis

Characteristic features of intestinal dysbiosis	
I-degree (compensated, latent form)	Qualitative and quantitative changes in the aerobic part of the microbiocenosis (increase or decrease in the content of E. coli) against the background of a slight decrease in bifid flora to 105 CFU/g. Detection of E.coli with atypical properties (lactose-negative, weakly fermenting). Identification of opportunistic flora (Klebsiella, Staphylococcus aureus, Proteus, fungi of the genus Candida).
II-degree (subcompensated form)	Moderate reduction in the content of bifidobacteria (up to 107-108 FU/g). Pronounced qualitative and quantitative changes in E.coli, opportunistic pathogens.
III-degree	A significant decrease in the level of bifid flora (105-107 CFU/g) with a sharp change in the quantity and properties of E. coli (detection atypical forms of E.coli in high concentrations, the appearance of hemolytic forms of E. coli). High level of opportunistic microflora) up to 105 CFU/g) with a tendency to form associations.
IV-degree	Bifidoflora is sharply reduced or absent (less than 105 CFU/g). Changes in E.coli levels. Increase in obligate, facultative opportunistic and pathogenic microorganisms in associations in high concentrations (107 CFU/g).

Table 4. Indicators of Vaginal Dysbiosis by Type and Severity

State of vaginal biocenosis	Main group (n=49)		Control group (n=32)	
	абс.	%	абс.	%
Normocenosis	11	22,45	29	90,63
absolute	2	4,08	13	40,63
conditional	9	18,37	16	50
Dysbiosis	38	77,55	3	9,37
moderate	18	36,73	2	6,25
- anaerobic	7	14,28	1	3,12
- aerobic	3	6,12	0	0
- mixed	8	16,32	0	0
expressed	20	40,82	0	0
- anaerobic	20	40,82	0	0

The use of real-time PCR made it possible to establish that bacterial vaginosis was detected in 76.7% of women. At the same time, in women of group 1 in 41 (83.6%) cases, in women from group 2 – in 19 (59.3%). At a further stage, we studied the intestinal microbiocenosis in women from a comparative perspective (Table 5).

Table 5. Comparative Data on Intestinal Microbiocenosis Among Examined Women

Compound	Specific content of microorganisms, Ig CFU/g feces (M ± m)	
	Main group (n=49)	Control group (n=32)
Bifidobacterium	5,3±0,2	7,42±0,1
Escherichia lactose positive	6,8±0,15	8,2±0,06
Escherichia lactose-negative	3,7±0,18	2,0±0,14
Escherichia weakly fermenting	4,2±0,29	3,13 ±0,15
Escherichia hemolytic	0,6±0,27	0,3±0,15
Staphylococcus	0,8±0,17	0,4±0,07
Proteus	1,1±0,24	0,7±0,21
Klebsiella	0,5±0,26	0,4±0,21
Candida	0,9±0,18	0,6±0,12

As can be seen from the presented data, all women have intestinal dysbiosis, characterized by a significant decrease in the intensity of Bifidobacterium colonization (5.3±0.2 Ig CFU/g versus 7.42±0.1 Ig CFU/g; P<0.05), Escherichia lactose positive (6.8±0.15 Ig CFU/g versus 8.2±0.06 Ig

CFU/g), against the background of a significant increase in Escherichia lactose negative (3.7±0.18 Ig CFU/g versus 2.0±0.14 Ig CFU/g), Escherichia hemolytic (0.6±0.27 Ig CFU/g versus 0.3±0.15 Ig CFU/g), Staphylococcus (0.8±0.17 Ig CFU/g versus 0.4±0.07 Ig CFU/g), Proteus (1.1±0.24 Ig CFU/g versus 0.7±0.21 Ig CFU/g), Klebsiella (0.5± 0.26 Ig CFU/g versus 0.4±0.21 Ig CFU/g) and Candida fungi (0.9±0.18 Ig CFU/g versus 0.6±0.12 Ig CFU/g) compared with data from the control group, and in 68% with an overwhelming growth of fungi of the genus Candida, and in 64% of cases with a predominance of yeast flora.

Thus, the vaginal and intestinal biotopes are closely related. One of the leading endogenous factors that can influence the development of dysbiotic processes in the vaginal microbiota is the altered composition of the intestinal microbiota, which dictates the need to correct the composition of the microflora of both the vagina and intestines in the early postoperative period in order to prevent infectious and inflammatory processes.

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