Features of the vagina microbiotope in patients with adenomyosis

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The objective: to assess the composition of the vaginal microbiota in women of reproductive age with adenomyosis.

Materials and methods. A comprehensive clinical-laboratory and instrumental prospective examination of 224 patients aged 30 to 50 years (average age – 42.0±1.8 years) with adenomyosis, who were included in the main study group, was conducted. The patients of the main group were divided into 2 subgroups depending on the form of adenomyosis – I group (diffuse form) included 106 women, II group (nodular form) – 118 persons. The control group included 84 healthy women of reproductive age with a regular ovulatory menstrual cycle and no history of gynecological diseases.

Patients underwent microscopic examination of vaginal secretions, bacterioscopic examination of vaginal smears, stained according to Gram. Lactobacillus cultures were identified by polymerase chain reaction (PCR) and 16S ribosomal ribonucleic acid analysis.

Results. During the microscopic examination of the vaginal secretion, more than 15 leukocytes in the field of view were detected in 84 (37.5±3.2%) patients of both groups: in 40 (47.9±3.3%) patients of the I group and in 44 (52.1±3.3%) – II group. During the bacterioscopic examination of vaginal swabs, stained according to Gram, no gonococci and trichomonads were found in the main group. Candida fungi were detected microscopically in 138 patients (70.5±3.1%) of the main group: in 71 (66.9±4.6%) of the I group and in 87 (33.1±4.3%) of II group.

Genotyping results showed that lactobacilli isolated from women with adenomyosis belonged to seven species: Lactobacillus fermentum, Lactobacillus iners, Lactobacillus gasseri, Lactobacillus amnionii, Lactobacillus jensenii, Lactobacillus crispatus, and Lactobacillus delbrueckii. The majority of lactobacilli strains have high inhibitory activity against all test strains, except for Candida albicans (39 cases, 46.4%). High adhesion to human erythrocytes was determined by 54.7% (46 cases) of the studied cultures. All lactobacilli strains were resistant to metronidazole, 83.3% (70 cases) to kanamycin, 57.1% (48 cases) to vancomycin, all strains were sensitive to roxithromycin, amoxiclav, and ampicillin. Moderate sensitivity to gentamicin and cefazolin was found in 51.9% (43 cases) of cultures, peroxide-forming activity in 80.9% (68 cases).

The use of quantitative diagnostic thresholds has led to significant improvements in diagnostic performance. PCR showed 88% sensitivity and 95% specificity for Gardnerella vaginalis and 96% sensitivity and 97% specificity for Atopobium vaginae.

Conclusions. It has been established that bacterial vaginosis and microorganisms associated with bacterial vaginosis are independently associated with adenomyosis. The results of the study confirmed that vaginal microorganisms, which are usually diagnosed in women with adenomyosis, ascend to the endometrium from the vagina vertically, which leads to infection of the upper parts of the genital tract.

Keywords: adenomyosis, microflora, diagnosis, uterus, differential diagnosis.
Adenomyosis is a common gynecological disease characterized by diffuse or local proliferation of endometrial glandular tissue, penetrating into the muscular layer of the uterus [1–3]. This condition is most often observed in women aged 30 to 50 years and is usually a consequence of an intrauterine procedure [4–7]. Characteristic symptoms of adenomyosis are menorrhagia, dysmenorrhea, uterine enlargement and infertility [8, 9]. The prevalence rate of adenomyosis ranges from 5% to 70%, depending on differences in ethnicity, case selection, and diagnostic criteria [10–13]. To improve the quality of life of patients, various therapeutic approaches are widely used, such as conservative treatment, surgery, hormonal therapy and interventional radiology or minimally invasive therapy [14–16].

Adenomyosis is the actual clinical problem in gynecology and health economics, since hysterectomy is most often used to treat premenopausal and perimenopausal women [17, 18]. Women with characteristic symptoms of adenomyosis and in most cases receiving treatment for adenomyosis are between the ages of 40 and 50 and are multiparous. However, the diagnosis is increasingly being made in young women who want to preserve their fertility [19, 20]. Thus, the development of minimally invasive and uterine-sparing treatments and the demand for these treatments requires a better understanding of the disease [21, 22].

In modern gynecology, one of the most relevant problems remains the problem of treating adenomyosis, a pathological condition disrupting menstrual and reproductive functions. The changes of microbiome and increasing of microbial richness was associated with adenomyosis [23]. The widespread uncontrolled use of antibacterial drugs leads to the selection of antibiotic-resistant strains of various pathogens of infectious diseases, which ultimately reduces the effectiveness of the main treatment [24–26]. The situation is complicated by the fact that the population also often uses these drugs unjustifiably.

In this regard, the purpose of our study was to assess the composition of the vaginal microbiota in women of reproductive age with adenomyosis.

**MATERIAL AND METHODS**

The study was conducted on the basis of Educational Surgery Clinics of Azerbaijan Medical University and Baku Health Center in the period from 2021 till 2023. The research was approved by Ethical Committee of Azerbaijan Medical University (protocol No 3; 04/03/2021)

In the course of this study, a comprehensive clinical, laboratory and instrumental prospective examination was carried out on 224 patients aged 30 to 50 years (average age – 42.0±1.8 years) with adenomyosis.

These patients constituted the main study group. Patients of the main group were divided into 2 subgroups depending on the form of adenomyosis (Group I – diffuse form – 106 women, Group II – nodular form – 118 women).

The control group consisted of 84 women of relatively healthy reproductive age (average age – 44.5±1.3 years), with a regular ovulatory menstrual cycle and without a history of gynecological diseases. Cohort studies are prospective. Inclusion criteria for the study: patient diagnosed with adenomyosis; patient age from 30 to 50 years; obtaining written consent from the patient to participate in examinations.

Exclusion criteria from the study: detection of a malignant pathological process in the organs of the reproductive system or other localization; the presence of hormone-synthesizing ovarian tumors; pregnancy and lactation; patients under 30 years of age and over 50 years of age; refusal to participate in the study.

The clinical characteristics of the examined women were based on the study of complaints, obstetric-gynecological and somatic anamnesis. All patients were examined for general and gynecological anamnesis and features of menstrual function. Particular attention was paid to previous inflammatory diseases of the genital organs, a history of spontaneous abortions, premature pregnancies, antenatal fetal death, the course of pregnancy, as well as their results. Patients in both groups underwent microscopic examinations of vaginal discharge and bacterioscopic examinations of Gram-stained vaginal smears. Vaginal smears were Gram stained for bacterial vaginosis using the standardized method described by Nugent et al. 54 Lactobacillus cultures were isolated from the vaginal epithelium of women with diagnosed adenomyosis and healthy women of reproductive age. Identification of cultures was carried out using PCR (Polymerase chain reaction) analysis of 16S ribosomal RNA (ribonucleic acid). Statistical analysis of the data was carried out using data from Microsoft Excel spreadsheet programs, generated in accordance with the requests of the ongoing research.

**RESULTS AND DISCUSSION**

Studies have found that the largest group consists of patients aged 36–40 years. There were 92 women (41.1±3.3%) of this age in the main group, and 37 (44.0±5.4%) in the control group.

When dividing patients into the main group, it was found that in the Group I there were 45 women (42.5±4.8%), in the Group II – 47 (39.8±4.5%) in the age group 36–40 years. The smallest group consisted of patients aged 30-35 years; the main group included 60 women of this age (26.8±2.9%). The age characteristics of the patients are presented in Table 1.
When dividing patients into the main group, it was found that in the age group of 30–35 years, 28 women of the Group I were identified, which is 26.4±4.3%, and 32 women of the Group II (27.1±4.1%). When dividing patients into main groups, it was found that in Group I there were 60 women of reproductive age, which is 26.8±2.9%, in group 2 – 92 women (41.1±3.3%). A total of 152 patients (67.9±3.1%) of the main group were of reproductive age, and 72 patients (32.1±3.1%) were of perimenopausal age.

A regular, ovulatory, adequately hormonally supported menstrual cycle is considered an indicator of the normal functioning of the reproductive system.

Therefore, the study of menstrual history in the study groups determines the possible difference between the groups in the duration of menstruation and the duration of the menstrual cycle (MC). At p<0.0001, there was a statistically significant difference in the age of menarche between the groups, while in Group I, the first menstruation begins at 9–11 years; in Group II – 10–13 years old (Table 2).

The data obtained established a significant difference in the duration of the menstrual cycle between patients with and without adenomyosis, which, undoubtedly, can serve as a criterion for choosing the severity of the stage of development of adenomyosis and its spread. Thus, the clearer the stage of adenomyosis, confirmed by histological examination, the earlier (9 years) menstruation begins, which can be noted as an unfavorable factor for determining the severity of the spread of adenomyosis and the time of its manifestation.

In patients with adenomyosis, menstrual irregularities such as hyperpolymenorrhea occur in almost every third patient, and algomenorrhea - in almost every second patient (Table 3).

From the above data it is clear that hyperpolymenorrhea occurred in 34.9±4.6% of patients in the Group I (37 women), in the Group II (28 women) – in 23.7±3.9%, and in the control group (9 women) was detected in 10.7±3.4%. Algodysmenorrhea was observed in groups accordingly: 49.1±4.8% (52 women), 27.9±4.1% (33 women) and 7.1±2.8% (6 women).

The duration of the disease is of great importance when conducting research on a particular nosology. From the moment of diagnosis of adenomyosis in our study, it took from 3 to 8 years, the average period of diagnosis was 6.2±1.4 years. It should be noted that, as a rule, the duration of diagnosis increases in parallel with the prevalence of adenomyosis. It has also been found that the lack of criteria for early diagnosis of adenomyosis also leads to delays and difficulties in diagnosis.

The laboratory method generally accepted as the gold standard for diagnosing bacterial vaginosis (BV) is microscopic examination of Gram-stained vaginal smears. Because both the Nugent scoring system and the Amsel criteria used in clinical practice have their limitations, DNA-based (deoxyribonucleic acid-based) assays targeting bacteria associated with the condition of BV are required for objective, reproducible, and accurate diagnosis of BV.

The diagnosis of BV was also determined by the clinician and included the mandatory presence of three Amsel criteria (increased pH, clue cells, and discharge with a fishy odor).

### Table 1

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<thead>
<tr>
<th>Age characteristics of the examined patients</th>
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<td>Groups</td>
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<tr>
<td>Group I (n=106)</td>
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<td>Group II (n=118)</td>
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<td>Total (n=224)</td>
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<td>Control group (n=84)</td>
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### Table 2

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<th>Characteristics of menstrual function in groups</th>
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<td>Indicators</td>
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<tr>
<td>Duration of menstruation/day</td>
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<td>Duration of menstrual cycle/day</td>
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Notes: * – р I-II<0.0001; ** – р II-III<0.0001.

### Table 3

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<th>Type of menstrual irregularities in the examined patients</th>
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<td>Indicators</td>
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<tr>
<td>Hyperpolymenorrhea</td>
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<td>Algidosmenorrhea</td>
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The studies found that more than 15 leukocytes in the field of view during microscopic examination of vaginal secretions were detected in 84 (37.5±3.2%) patients of both groups: in 40 (47.9±3.3%) patients of Group I and in 44 (52.1±3.3%) women of Group II. Bacterioscopic examinations of Gram-stained vaginal smears carried out in the study groups did not reveal gonococci and trichomonas. Fungi of the genus Candida were microscopically detected in 158 patients (70.5±3.1%) of both groups: in 71 (66.9±4.6%) of Group II and in 87 (33.1±4.3%) of Group I.

Genotyping results showed that in women without adenomyosis Lactobacillus spp., Lactobacillus gasseri and Lactobacillus jensenii are the dominant species of vaginal microbiome. In women with adenomyosis seven species of lactobacilli were revealed: Lactobacillus fermentum, Lactobacillus iners, Lactobacillus gasseri, Lactobacillus amnionii, Lactobacillus jensenii, Lactobacillus crispatus and Lactobacillus delbrueckii. Most strains of lactobacilli have high inhibitory activity against all test strains, except Candida albicans (39 cases, 46.4%). 54.7% (46 cases) of the studied cultures showed high adhesion to human erythrocytes. All strains of lactobacilli were resistant to metronidazole, 83.3% (70 cases) – to kanamycin, 57.1% (48 cases) – to vancomycin, all strains were diagnosed with sensitivity to roxithromycin, amoxiclav, ampicillin. 51.9% (43 cases) of cultures showed moderate sensitivity to gentamicin and cefazolin. 80.9% (68 cases) of cultures exhibited peroxide-producing activity.

The test performed gave positive or negative results for the following three diagnoses:

1) bacterial vaginosis (BV) by algorithmic analysis of molecular DNA detecting lactobacilli, as well as bacteria associated with BV, Gardnerella vaginalis, Atopobium vaginae, Megasphaera sp.;
2) vaginal candidiasis by detecting molecular DNA of the Candida group (Candida albicans, Candida tropicalis, Candida parapsilosis and Candida dubliniensis), Candida glabrata or Candida krusei.
3) trichomoniasis using molecular DNA detection of Trichomonas protozoa.

The most studied vaginal anaerobe, Gardnerella vaginalis, has been recovered from vaginal specimens of almost all women with BV.

Qualitative indicators for bacterial detection using species/genus-specific PCR assays are presented as follows: Lactobacillus iners was the most frequently detected bacterium in women with adenomyosis (86.9%, 73 cases), followed by Gardnerella vaginalis (80.9%, 68 cases), Atopobium vaginae (73%, 61 cases), Prevotella spp. (73.8%, 62 cases), Mobiluncus mullieri, (70.2%, 59 cases), Finegoldia magna (57.1%, 48 cases), Lactobacillus amnionii (51.2%, 43 cases), Eggerthella spp. (46.4%, 39 cases) and Sneathia sanguinigenes (42.9%, 36 cases). Mycoplasma bacteria, including Mycoplasma hominis and Ureaplasma urealyticum, have also been isolated from the upper genital tract of women with adenomyosis. In BV, the number of lactobacilli decreases with an increase in the number of microorganisms such as Atopobium vaginae, Gardnerella vaginalis, Mobiluncus mullieri, Prevotella spp. and Megasphaera sp.

The use of quantitative diagnostic thresholds has led to improvements in diagnostic performance. PCR showed 88% sensitivity and 95% specificity for Gardnerella vaginalis and 96% sensitivity and 97% specificity for Atopobium vaginae. Bacterial vaginosis, diagnosed using Nugent’s criteria, has been associated with adenomyosis in women. We found that bacterial vaginosis and bacterial vaginosis-associated microorganisms are independently associated with adenomyosis.

The results of our research demonstrated that there are differences in the composition of the microflora of the vagina in women with and without adenomyosis.

In the other study there were found out the similar data. Kunaseth J. et al. (2022), using linear discriminant analysis effect size technique indicated an elevated abundance of several vaginal microorganisms in the women with adenomyosis (Alhoscardovia, Oscillospirales, Ruminococcaceae, Oscillospiraceae, Eihydrobacter, Megamonas, Moraxellaceae, Subdoligranulum, Selenomonadaceae, and Faecalibacterium). At the same time, the authors revealed an increase in the abundance of Megasphaera, Fasidiosipila, Hungateiclostriidaeae, and Clostridia in the control group without adenomyosis [23].

L. V. Pakhareno et al. (2023) revealed the different composition of the microflora in the women with and without endometriosis not only in vagina, but in the cervical mucus, uterine cavity and peritoneal fluid. According to their data, in women with endometriosis the microflora composition of the vagina manifested by a decreased concentration of Lactobacillus and the presence of a variety of other microorganisms in relation to healthy women. Despite the fact that pathology of the study was different, this research supports the theory of importance of vagina microbiome in development of various gynecological diseases [27].

R. J. Joseph et al. (2021) in their review summarized the latest findings about features of vaginal microbiome from several researches noted that there are advanced molecular tools to monitor and evaluate the extent of dysbiotic changes compared to a healthy state. They emphasized the high effectiveness of DNA-based methods to detect the composition of vagina microbiome [26]. We used the methods mentioned above that supports the significance of our data.

CONCLUSIONS

1. The results of our study support molecular studies showing that BV is associated with significant changes in the composition of the vaginal microbiota. Bacteria associated with BV, Gardnerella vaginalis, Atopobium vaginae, Eggerthella, Prevotella, Megasphaera, detected at diagnostic levels, expressed as the relative abundance or absolute quantity of the bacterial genome/gene (the number of genomes/copies of the 16S rRNA gene), were highly predictable.

2. Reduction of Lactobacillus species in combination with the presence of Gardnerella vaginalis or Atopobium vaginae at the diagnostic level was a very accurate predictor of BV. Measuring the amount of normal vaginal microbiota relative to the total number of bacteria in vaginal secretions may provide more accurate tools for diagnosing BV.

3. Our results indicate that vaginal microorganisms, commonly found in women with adenomyosis, ascend from the vagina to the endometrium, leading to upper genital tract infection.
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