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Original Article

The state of genital microbiocenosis in women with functional ovarian cysts on the background of type 2 *diabetes mellitus*

Olga Sergeevna Shapoval [1] *, Michael Ivanovich Sheremet [2]

[1] Department of Gynecology, Zaporizhian Medical Academy of Postgraduate Education of the Ministry of Healthcare of Ukrain, Zaporizhia, Ukraine

[2] Surgery Department No. 1, Bukovinian State Medical University, Chernivtsi, Ukraine

* Correspondence to: Shapoval Olga Sergeevna, Department of Gynecology, Zaporizhian Medical Academy of Postgraduate Education of the Ministry of Healthcare of Ukraine, Vinter Avenue 20, Zaporizhzhia, Ukraine. Phone: +380508195300; E-mail: shapoval_olga@ukr.net

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Abstract

The aim of the work is to study the state of genital biocenosis in women with functional ovarian cysts on the background of type 2 *diabetes mellitus*. Sixty patients of reproductive age with functional ovarian cysts and type 2 *diabetes mellitus* were examined in the Department of Gynecology in the Limited liability company "VITACENTER". The control group was represented by 50 healthy non-pregnant women. General clinical, ultrasound examination of pelvic organs and bacteriological and molecular genetic study of discharge from the genital tract was carried out. The survey found in 40–65% of patients the activation of their own conditionally pathogenic flora. There were diagnosed 25% cases of normocenosis of the vagina and 75% cases of dysbiosis of the vagina. Qualitative and quantitative assessment of the microbiome of the genital tract is fundamentally necessary for understanding the strategy of treatment of pathological conditions in gynecological practice. The spectrum of the most aggressive microflora prevailed in patients with infertility, which contributed to the reduction of nonspecific reactivity and the maintenance of the existing inflammatory process.

Keywords: type 2 *diabetes mellitus*, functional ovarian cysts, microbiocenosis.

Introduction

Nowadays, the state of the genital biotope is considered one indicator of women's reproductive health. Qualitative and quantitative assessment of complex microbial communities becomes absolutely necessary for diagnosing dysbiotic disorders caused by conditionally pathogenic microorganisms in most cases.

As it is known, the normal vaginal microbiota is represented by both aerobes and anaerobes. *Lactobacillus* provides immunological protection by maintaining the acidic pH of the vagina and the synthesis of hydrogen peroxide in the mucous membrane [1].

Changes in the number of microorganisms in a biotope, or the appearance of bacteria not peculiar to this habitat, serve as a signal of adaptive or irreversible changes in the corresponding link of homeostasis [2].

Thus, quantitative and qualitative assessment of complex microbial communities becomes absolutely necessary for diagnosing dysbiotic disorders caused by both commensals and representatives of pathogenic flora.

The aim of the work

The article is aimed to study the state of genital biocenosis in women with functional ovarian cysts on the background of type 2 *diabetes mellitus*.

Material and methods

Sixty patients of reproductive age with functional ovarian cysts and type 2 *diabetes mellitus* were examined in the Department of Gynecology in the Limited

liability company "VITACENTER" for the period 2019–2021 years. The control group was represented by 50 healthy non-pregnant women aged (29.32±1.07) years who were consulted about their contraception.

According to existing standards, all patients underwent a general clinical examination and gynecological examination. Ultrasound examination of the pelvic organs showed the presence of tumor-like formation of the ovaries size from 3 to 5 cm.

All the patients in the main group had type 2 *diabetes mellitus*. The disease was at the stage of compensation. The average level of HbA1c was (5.8±0.21)% due to taking Metformin hydrochloride 1500 mg daily.

An obstetrician-gynecologist carried out the microbiological examination of vaginal discharge and cervix by sampling the material from the vagina with a sterile cotton swab. The material was sifted into 5% blood agar and the Endo by the Gold method to count the growing colonies in the laboratory. Crops were placed in a thermostat at 37°C for 24–72 hours. When colonies were found on nutrient mediums, their counting and sifting into sectors of nutrient media and simple, nutritious agar for the identification of crops was carried out (the indicator of microbial seeding was determined by colonizing units/ml).

For the completeness of the assessment of the state of biocenosis of a urogenital additional method of polymerase chain reaction in real time using femoflor®SKRIN reagents, DT-96 detective amplifier, DNA-technology production DT Prime (DNA TECHNOLOGIES).

The state of microbiocenosis was evaluated by comparing the amount of normal microflora (*Lactobacillus spp.*) with the total bacterial mass. The absence of significant differences between these indicators testified to the preservation of norm flora. As a rule, a significant decrease in the number of lactobacteria in relation to the total bacterial mass was accompanied by sexually transmitted diseases. It also indicated dysbiotic disorders of varying severity, in which the number of conditionally pathogenic bacteria increased against the background of a decrease in normal microflora. Accounting and interpretation of reaction results were carried out automatically using the software.

Statistical analyses were performed using the software package STATISTICA (StatSoftStatistica v.6.0).

Results

The average age in the main group was (30±0.73) years. Depending on the reproductive function, they were allocated to 3 groups of patients: with infertility – 16 (26.67%) women, women who did not give birth – 15 (25%), who gave birth – 29 (48.33%).

It was found that 13 patients (21.67%) had a relapse of functional ovarian cysts. Eleven women (84.62%) were operated on due to rupture of the cyst in the past. Six (46.15%) patients had the experience of using combined oral contraceptives.

Thirty-three patients (55%) had chronic inflammation of the uterus and in 8 cases, hydrosalpinxes took place during hysterosalpingography.

During the gynecological examination of patients with functional ovarian cysts, the presence of exocervicitis in 42 women (70%) and endocervicitis in 12 women (20%) was established during colposcopy. In the control group, a clean pink cervical mucosa was found. In 20 women with ovarian cysts (33.33%), abundant vaginal discharge of a yellow-milky color in 13 cases (65%) and green color in 7 cases (35%) were diagnosed. In women from the control group, vaginal discharge was moderate and transparently white.

Microscopy of the vaginal secretion in women of the main group revealed from 1 to 35 leukocytes in the field of view. In 6 cases (10%), the number of leukocytes was from half to the entire field of view. In 42 patients (70%), a high level of epithelial elements was found and *Candida* and *Mobiluncus* dominated. Single leukocytes and epithelial cells, *Lactobacillus*, were established in all cases in women of the control group.

In patients with ovarian cysts, regardless of the previously implemented generative function, the sown flora presented by *Str. pyogenes*, *S. epidermidis*, *S. aureus*, *E. coli*, *C. albicans*. *Str. pyogenes* was determined in a concentration of more than 10^6 CU/ml in every third patient and in moderate concentration (10^4–10^6 CU/ml) in every seventh woman. In every third patient was determined *S. epidermidis* in moderate concentration (10^4–10^6 CU/ml). In every fourth patient were determined *S. aureus* and *Ent. faecalis* in moderate concentration (10^4–10^6 CU/ml). In 25.96% of patient's concentration the presence of *E. coli* was found to be more than 10^6 CU/ml (Table 1).

The study of the microbial biotope of the genital tract found the presence of *C. albicans* in low and medium concentrations in 32.69% of patients. In 34% of cases, associations of *E. coli* and *C. albicans* were diagnosed in vaginal discharge in the group of patients with infertility and ovarian cysts and 25% associations of *S. epidermidis* and *C. albicans*.

Along with the increase in the colonization of optional microflora in women of the main group, there

Table 1: Indicators of the microflora of the genital tract in women of reproductive age with functional ovarian cysts and *diabetes mellitus*.

Type of microorganism	Concentration, CU/ml	Control group n=50		Patients with ovarian cysts and *diabetes mellitus* n=60	
		n	%	n	%
1	2	3	4	5	6
	10^2–10^4	4	8	2	3.33
Str. Pyogenes	10^4–10^6	1	2	9	15
	>10^6	0	0	18	30
	10^2–10^4	2	4	6	10
S. epidermidis	10^4–10^6	1	2	19	31.67
	>10^6	-	-	2	3.33
	10^2–10^4	5	10	2	3.33
S. aureus	10^4–10^6	2	4	15	25
	>10^6	-	-	7	11.67
	10^2–10^4	1	2	7	11.67
Ent. Faecalis	10^4–10^6	-	-	15	25
	>10^6	-	-	2	1.2
	10^2–10^4	4	8	3	5
E. coli	10^4–10^6	-	-	8	12.5
	>10^6	-	-	15	25
	10^2–10^4	3	6	12	20
C. albicans	10^4–10^6	2	4	7	11.67
	>10^6	-	-	-	-

was a decrease in the intensity of colonization of *Lactobacillus spp*. The condition of normocenosis, in which the share of lactobacilli relative to the total bacterial mass was more than 80%, was diagnosed in 25% of the cases. Seventy-five% of the patients with ovarian cysts and *diabetes mellitus* had a state of vaginal dysbiosis. Moderate dysbiosis (the lactobacilli share ranges from 20% to 80%) was diagnosed in 41.67% of patients. Severe dysbiosis (the share of lactobacilli less than 20%) was found in 33.33%. In the control group, the total bacterial mass and the number of Lacto bacteria fluctuated significantly – from 10^5 to 10^9 CU/ml. At the same time, the share of lactobacilli amounted to about 80% in healthy women.

In patients with ovarian cysts, *Ureaplasma spp*. was found in 24 (40%) of patients and *Gardnerella vaginalis* – 39 (65%) of patients, which indicated immune dysfunction. The concentration of *Ureaplasma spp*. in 62.5% of patients was medium (10^4–10^6 CU/ml) and in 29.17% of cases, it was high (>10^6 CU/ml). In 51.28% of cases, the average concentration of *Gardnerella vaginalis* (10^4–10^6 CU/ml) was determined in 41.03% – a high titer (>10^6 CU/ml). In 68.42% of cases identified, microbial agents were diagnosed in patients with infertility and women who did not give birth. *Mycoplasma spp*. was found in 13 patients (21.67%) and *Chlamydia trachomatis* in 14 (23.33%) patients at high and medium concentrations (Table 2).

The concentration of lactobacilli ranged from 80% to 100%, which was regarded as a manifestation of normocenosis in the control group. The concentration of *Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp*. ranged from 10^2 to 10^4 CU/ml, corresponding to less than 1% of the total bacterial mass. Representatives of *Mycoplasma* also were not determined. In 10 cases (20%) in the control group, *Candida* was found in an amount not higher than 10^3 CU/ml. However, the clinical manifestation of fungal infection was not detected (Tables 1 and 2).

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Table 2: Detectability of sexually transmitted infections in patients of reproductive age with functional ovarian cysts and *diabetes mellitus*.

Type of microorganism	Concentration, CU/ml	Control group n=50		Patients with ovarian cysts and *diabetes mellitus* n=60	
		n	%	n	%
Ureaplasma spp.	10^2–10^4	-	-	2	8.33
	10^4–10^6	-	-	15	62.5
	>10^6	-	-	7	29.17
Mycoplasma spp.	10^2–10^4	-	-	1	7.69
	10^4–10^6	-	-	10	76.92
	>10^6	-	-	2	15.38
Chlamydia trachomatis	10^2–10^4	-	-	-	-
	10^4–10^6	-	-	-	-
	>10^6	-	-	14	100
Gardnerella vaginalis	10^2–10^4	5	10	3	7.69
	10^4–10^6	-	-	20	51.28
	>10^6	-	-	16	41.03

Discussion

Benign tumor-like formations of the ovaries are diagnosed in 19–25% of patients with ovarian tumors [3, 4]. Ovarian cysts affect patients of different ages, but it is very important to solve the problem of timely diagnosis and quality treatment in patients of reproductive age, especially those with companion endocrine pathology. Waiting tactics, chaotic administration of hormonal drugs, the development of chronization of the process, conjugation and infertility contribute to an increase in the frequency of surgical aggression in patients with ovarian pathology. It leads to a decrease in ovarian reserve and reproductive potential [5–9].

That is why the aim of the work was to study the state of genital biocenosis in women with functional ovarian cysts on the background of type 2 *diabetes mellitus*.

It was revealed that in 21.67% of cases, there was a relapse of functional ovarian cysts. 55% of patients suffered from chronic inflammatory processes of the uterus and appendages, treatment of which was previously performed without taking into consideration the etiologic factor. The conducted examination revealed 40–65% of patients' activation of their own conditionally pathogenic flora. There were diagnosed 25% cases with normocenosis of the vagina and 75% cases with dysbiosis of the vagina. The spectrum of the most aggressive microflora prevailed in patients with infertility, which contributed to a decrease in nonspecific reactivity and maintenance of the existing inflammatory process.

It is believed that one of the causes associated with impaired vaginal biocenosis is the incapable immunological homeostasis [10, 11] which is also characteristic of *diabetes mellitus*. On the one hand, violations of the biotope of the genital mucosa cause suppression of local immunity, but on the other hand, against the background of reduced immunological protection, conditions arise for the implementation of the pathogenic influence of commensals. This leads to a decrease in immunological insolvency of the body [10–12].

Nowadays, along with conditionally pathogenic microorganisms, the decisive role belongs to sexually transmitted diseases such as chlamydiosis, mycoplasmosis and ureaplasmosis with minimal clinical features [13]. This feature is due to the peculiarities of the microbial agent's interaction with the human body's immune system, namely the possible development of autoimmune reactions as a result of mitogenic effects on lymphocytes, as well as a direct immunosuppressive effect on the mechanisms of local immunity [2].

The launched cascade of reactions leads to the increase of proinflammatory cytokines and growth factors which leads to the development of proliferative processes [14, 15] and changes the expression of receptors to steroid hormones [16].

In patients with ovarian cysts, *Ureaplasma spp.* was found in 40% of patients and *Gardnerella vaginalis* in

65%, which indicated immune dysfunction. In 68.42% of cases identified, microbial agents were diagnosed in patients with infertility and women who did not give birth. *Mycoplasma spp*. was found in 21.67% and *Chlamydia trachomatis* in 23.33%. In 68.42% of cases, detectable infectious agents were diagnosed in patients with infertility and women who did not give birth.

In 34% of cases in the group of patients with infertility and ovarian cysts, the association of E. coli and C. *albicans* was diagnosed in vaginal discharge and in 25% of cases, the association of S. *epidermidis* and C. *albicans*. This fact leads to the reduction of nonspecific reactivity and maintenance of the existence of both *diabetes mellitus* and inflammatory process and can be counted as one of the reasons for infertility.

Thus, the results of a comprehensive study of microbial biocenosis in women with functional ovary cysts and *diabetes mellitus* show the significant role of the infectious factor in the development of ovarian pathology. Changes in biotope in the form of mixed infections have been identified, during which microbial associations actively interact with the saprophytic autoflora of patients with *diabetes mellitus*, and the concept of chronic diseases as infections of biofilms significantly complicates the process of effective treatment of this group of patients.

Conclusions

Sexually transmitted diseases were found in 40–65% of women with functional ovarian cysts and type 2 *diabetes mellitus*. Their own conditionally pathogenic flora is detected. There were diagnosed 25% cases with normocenosis of the vagina and 75% cases with dysbiosis of the vagina. The rate of the most aggressive microflora is determined in patients with infertility, which reduces nonspecific reactivity and maintains the existing inflammatory process. Bacterial and bacterial-fungal associations lead to inversion and the absence of a typical clinical picture of the disease and the formation of asymptomatic forms of the pathological process.

Conflict of interest

The authors declare no conflict of interest.

References

1. Baranovskaya, G.A. (2012). Peculiarities of local immunity in infectious and inflammatory diseases of the vagina. Women's health. 7 (73): 148-151.
2. Shapoval, O.S., Vorontsova, L.L. (2015). The state of functional and metabolic activity of phagocytes in women of reproductive age with tumor-like ovarian. Laboratory diagnostics. Eastern Europe. 2 (14): 64-70.
3. Vovk, I.B., Vdovychenko, Y.P., Kornatska, A.G. (2017). Tumor-like lesions of the ovaries. Kyiv,. 242 p.
4. Gerasimova, T.V. (2014). Optimization of diagnostics and treatment of functional ovarian cysts. Medical aspects of women's health. 5 (80): 65-73.
5. Matsuzaki, S., Houlle, C., Darcha C. (2009). Analysis of risk factors for the removal of normal ovarian tissue during laparoscopic cystectomy for ovarian endometriosis. Hum. Reprod. 24: 1402-1406.
6. Hassa, H., Tanir, H.M., Tekin, B. (2009). Apoptosis patterns in eutopic and ectopic endometrium, adhesions and normal-looking peritoneum from women with or without endometriosis. Arch Gynecol Obstet. 280 (2): 195-199.
7. Brosens, I., Gordts, S., enagiano, G. (2013). Endometriosis in adolescents is a hidden, progressive and severe disease that deserves attention, not just compassion. Human Reproduction. 28: 2026-2031.
8. Guo, S.W. (2009). Recurrence of endometriosis and its control. Hum. Reprod. Update. 15: 441-461.
9. Zhenyue, H., Tak W. Mak (2010). Type I and Tape II Pathways of FAS-mediated Apoptosis Are Differentially Controlled by XIAP. L of Molec Cell Biology. 2, 2: 63-64.
10. Tilly, J.L. (2004). The role of apoptosis in the development, functioning and dysfunction of the ovaries. The Ovary. 19: 321-345.
11. Verhelst, R., Verstraelen, H, Claeys G. (2004). Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between Atopobium vaginal, *Gardnerella vaginalis* and bacterial vaginosis. BMC microbiology. 4: 16.
12. Vdovichenko, Yu.P., Gopchuk E.N. (2013). Bacterial vaginosis and nonspecific vaginitis - rational therapy. Women's health. 1 (77): 149-154.
13. Ravel, J., Gajer, P., Abdo, Z. (2011).Vaginal microbiome of reproductive-age. Proc Natl Acad Sci USA. 108 (1): 4680-4687.
14. Kuznetsova L.V., Babadzhan V.D., Vorontsova L.L. (2013). Immunology. Vinnitsa, «Merciuri-Podillya», 564 p.
15. Kazmirchuk, V.E., Kovalchuk, L.V., Maltsev, D.V. (2012). Clinical immunology and allergology with age-related characteristics: Kyiv, «Medicine», 520 p.
16. Tatarchuk, T.F. (2010). Inflammatory diseases of pelvic minor: when the treatment should be started immediately. Women's health. 2: 52-53.

https://doi.org/10.46389/rjd-2022-1161