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НОВЫЙ ДЕНЬ В МЕДИЦИНЕ
NEW DAY IN MEDICINE**

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ANALYSIS OF THE MICROBIOTOPE OF CERVICOVAGINAL DISCHARGE IN WOMEN WITH CHRONIC CERVICITIS AND SQUAMOUS CELL INTRAEPITHELIAL LESIONS OF THE CERVIX

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✓ **Resume**

In women with chronic cervicitis, the composition of cervicovaginal discharge is predominantly represented by associations of microorganisms (93.8%): bacterial (44.9%) and bacterial-viral (55.1%). With the development of cervical SIL, the composition of microorganisms in cervicovaginal discharge significantly changes: the number of bacterial-viral associations increases (59.6%), in which the prevalence of high-risk HPV is 52.9%.

Keywords: cervix, human papillomavirus, intraepithelial neoplasia.

АНАЛИЗ МИКРОБИОТОПА ЦЕРВИКОВАГИНАЛЬНОГО ОТДЕЛЯЕМОГО У ЖЕНЩИН С ХРОНИЧЕСКИМ ЦЕРВИЦИТОМ И ПЛОСКОКЛЕТОЧНЫМИ ИНТРАЭПИТЕЛИАЛЬНЫМИ ПОРАЖЕНИЯМИ ШЕЙКИ МАТКИ

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✓ **Резюме**

Женщин с хроническим цервицитом состав цервиковагинального отделяемого представлен преимущественно ассоциациями микроорганизмов (93,8%): бактериальными (44,9%) и бактериально вирусными (55,1%). При развитии SIL шейки матки состав микроорганизмов цервиковагинального отделяемого достоверно изменяется: увеличивается число бактериально вирусных ассоциаций (59,6%), в которых распространенность ВПЧ высокого онкогенного риска составляет 52,9%.

Ключевые слова: шейка матки, вирус папилломы человека, внутри эпителиальная неоплазия.

SURUNKALI SERVISIT VA BACHADON BO'YNI YASSI HUYAYRALI INTRAEPITELIAL SHIKASTLANISHLARI BO'LGAN AYOLLARDA BACHADON BO'YNI VAGINAL AJRALMA MIKROBIOTOPINING TAHLILI

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✓ *Rezyume*

Surunkali servitsit bilan og'rigan ayollarda servikal vaginal ajralma tarkibi asosan mikroorganizmlar (93,8%) assotsiatsiyalari bilan ifodalanadi: bakterial (44,9%) va bakterial-virusli (55,1%). Servikal SIL rivojlanishi bilan servikal vaginal ajralmadagi mikroorganizmlarning tarkibi sezilarli darajada o'zgaradi: bakterial-virusli assotsiatsiyalar soni ortadi (59,6%), bunda yuqori xavfli HPV tarqalishi 52,9% ni tashkil qiladi.

Kalit so'zlar: bachadon bo'yni, inson papillomavirusi, intraepitelial neoplaziya.

Relevance

Inflammatory processes of the lower genital tract are the most common reason for women to consult an obstetrician-gynecologist and account for 54-76% of all gynecological diseases [1,3,5,7,9]. Inflammatory diseases of the cervix in the female population occur with a frequency of up to 40% and occur predominantly in a chronic, asymptomatic form [2,4,6,8,10]. According to modern publications, 80% of cervicitis is caused by microbial associations [3,11, 13, 14], with Chlamydia trachomatis being the leading initiator of inflammation (30-37%). The role of bacteria of the genus Staphylococcus (53-56%), Streptococcus (33%), opportunistic flora and anaerobes (20-56%) [2,19], viruses (7-80%), including HPV (5-86%) [1,12, 15, 16,17] has also been determined. It is known that the violation of the morphofunctional properties of tissues due to inflammatory processes with a chronic course is an important factor in the development of neoplasia [1,18]. Thus, the results of a number of studies have shown that cervicitis associated with bacterial and viral infections contribute to the development of cervical dysplasia and Ca in situ [1, 19,20]. However, this issue remains unresolved, as many authors focus on recognizing only the main role of oncogenic HPV types in the pathogenesis of cervical dysplasia and cancer [1,2,3] and refute reports of the involvement of bacterial and other viral infections in the development of neoplastic processes of the cervix [18, 21,22]. Modern scientific developments and technologies allow the use of various methods for diagnosing cervical cancer (CC), such as simple cytological examination, liquid oncocytology, immunocytochemical examination, colposcopy, histological and immunohistochemical examination of cervical tissues. However, despite the widespread implementation of screening programs, the detection of cervical neoplasia in the early stages of the disease remains insufficiently effective – according to the GLOBOCAN 2018 statistical service, cervical cancer ranks 4th in the world in the structure of malignant neoplasms in women [2,3,21].

Unfortunately, immunohistochemical (IHC) analysis of molecular biological markers, which largely underpin modern strategies for early cancer diagnosis, has not yet been widely used in gynecological practice. Therefore, it is necessary to develop approaches to an accessible and reliable set of sequential, targeted, personalized methods for assessing cervical health that will enable timely risk prediction and early detection of squamous intraepithelial lesions (SIL) of the cervix in women with chronic cervicitis.

Study Objective: To study the risk factors and developmental characteristics of SIL in women with chronic cervicitis in relation to a personalized management strategy.

Study Material and Methods

A clinical study included 235 female patients residing in the city of Bukhara and districts of the Bukhara region (BR), aged 18 to 35 years, who visited antenatal clinics from 2023 to 2025 and were diagnosed with mild cervical intraepithelial neoplasia associated with various types of HPV (HCR), without abnormal colposcopic findings. All patients underwent basic cervical diagnostic methods, including traditional cytological method, its modification (liquid-based cytological analysis); HPV testing and colposcopy; screening for sexually transmitted infections using PCR; ELISA for determination of HSP-60 IgG Ch. trachomatis; and the oncoprotein p16ink4 α . All patients underwent basic cervical diagnostic methods, including traditional cytological method, its modification (liquid-based cytological analysis); HPV testing and colposcopy; screening for sexually transmitted infections using the PCR method; ELISA for determination of HSP-60 IgG Ch. trachomatis, as well as cytological examination of the prognostic and diagnostic marker of disease progression – the oncoprotein p16ink4 α .

A total of 235 women were diagnosed with mild cervical intraepithelial neoplasia associated with various HPV types, without abnormal colposcopic findings. Results of cytological screening, HPV testing, colposcopy, and immunocytochemical determination of molecular tumor marker expression levels after 12 months of follow-up determined the distribution of the 235 patients into Group 1 and

Group 2. Group 1 included 60 HPV-positive patients (mean age 27.4 ± 5.1 years) with mild cervical lesions accompanied by tumor marker expression. Colposcopic examination in these patients revealed subtle lesions, such as thin acetowhite epithelium, delicate mosaicism, punctuation, or a combination of these. Group 2 included 175 patients (mean age 26.2 ± 6.4 years) with LSIL and no expression of the p16ink4 α oncoprotein. The control group consisted of 60 apparently healthy women who were negative for HPV-associated cervical lesions based on cytological screening at their initial visit. Patients were selected for the study by stratified randomization according to the inclusion and exclusion criteria. Inclusion criteria: written informed consent and ability to undergo all tests; HPV-positive valid test result; verified diagnosis of LSIL by cytological screening, confirmed by two methods (cervical scraping examination using the classic Pap test and liquid-based cytology); absence of colposcopic changes; age from 18 to 35 years; no HPV vaccination. The control group consisted of women with no pathological changes in the cervix, generally healthy women. Exclusion criteria: pregnancy and lactation; history of surgical interventions on the cervix for neoplasia in the indication for surgical treatment of HPV-associated SIL; use of immunomodulatory and antiviral drugs within the past 12 months; HPV vaccination; HIV infection, current detection of STIs; other acute or chronic diseases in the decompensation stage that affect the patient's general condition during the study period, the results of any laboratory and instrumental diagnostic methods specified in the study design, or the patient's compliance with the study.

Results and discussion

All women (n=57) with squamous cell intraepithelial lesions of the cervix were positive for one or more infectious agents (obligate pathogens and opportunistic pathogens). Analysis of the microbial composition of cervicovaginal discharge in all 57 women with SIL revealed associations of microorganisms (bacterial and bacterial-viral). Bacterial-viral associations were detected in 59.6% (n=34) of women with SIL, with isolated bacterial associations occurring more than 1.4 times less frequently in women with SIL—in 40.4% (n=23). It was found that obligate pathogenic infectious agents predominated in the microbial composition of cervicovaginal discharge in women with chronic cervicitis in the HSIL group compared to the LSIL and comparison group ($p < 0.05$). Moreover, in the HSIL group, obligate pathogenic flora was 3.6 times more common than in women without squamous intraepithelial lesions of the cervix. Women with NIL had significantly more opportunistic flora compared to the LSIL and HSIL groups ($p < 0.05$). Chlamydia trachomatis was three times more common in women with SIL compared to women in the NIL group ($p < 0.05$). Chlamydial infection occurred with comparable frequency in the NIL and HSIL groups ($p > 0.05$). According to the study results, one in two women with detected Ca in situ was infected with Chlamydia trachomatis. An analysis of the structure of causative agents of obligate infection revealed that Mycoplasma genitalium was found to infect 8.3% (n=3) of women in the LSIL group and 38.1% (n=8) of women in the HSIL group. Mycoplasma genitalium was not detected in the comparison group (NIL). Women with SIL of the cervix were found to be infected with Trichomonas vaginalis three times more often (14.0% (n=8)) than women in the comparison group ($p < 0.05$). Moreover, in the LSIL group, T. vaginalis was found 1.7 times more often than in women from the HSIL group ($p < 0.05$).

Women with SIL had a significantly higher incidence of obligate pathogenic infectious agents (59.6%) compared to women without cervical squamous intraepithelial lesions (26.1%) ($p < 0.05$). Depending on the severity of cervical squamous intraepithelial lesions, women in the HSIL group had a significantly higher incidence of M. genitalium compared to women in the LSIL and NIL groups ($p < 0.05$). Chl. trachomatis and T. vaginalis were isolated from one woman with Ca in situ. Gonococcal infection was not detected in any of the study groups. Analysis of the structure of opportunistic microflora revealed opportunistic mycoplasmas (U. urealyticum and/or M. hominis) in 35.1% (n = 20) of women with cervical squamous intraepithelial lesions. U. urealyticum was detected in 33.3% (n=12) of women in the LSIL group, 4.7% (n=1) of women in the HSIL group, and 26.0% (n=6) of women in the comparison group. M. hominis was detected in 13.9% (n=5) of women in the LSIL group, 9.5% (n=2) of women in the HSIL group, and 17.4% (n=4) of women in the comparison group. Women with chronic cervicitis and LSIL/NIL had a significantly higher rate of opportunistic mycoplasma infection than women with HSIL ($p < 0.05$). One woman (n=1) with Ca in situ was infected with U. urealyticum, and the other (n=1) with M. hominis. Women with HSIL were more likely to have Enterococcus faecalis

compared to women with LSIL and women in the comparison group, and *Candida albicans* ($p < 0.05$) compared to women with LSIL. Among women with Ca in situ, *Staphylococcus aureus* and *Candida albicans* were detected in one woman. A study of the microbial composition of the cervicovaginal discharge from women with Ca in situ revealed *Chl. trachomatis*, *U. urealyticum*, *T. vaginalis*, and *Candida albicans* in one woman, and *M. hominis* in another.

When analyzing the viral infection pattern, herpes simplex virus type II was detected in 3.5% ($n=2$) of women with SIL. However, it was not detected in two women in the LSIL group, in the HSIL group, and in only one woman in the comparison group. Herpes simplex type I was not detected in any women. Cytomegalovirus was detected in three women with LSIL and one woman with HSIL. Human papillomaviruses from the low-oncogenic risk group were significantly more frequently ($p < 0.05$) detected in women with chronic cervicitis in women with LSIL, while human papillomaviruses from the high-oncogenic risk group were significantly more frequently ($p < 0.05$) detected in women with HSIL. Two women with Ca in situ were found to have high-oncogenic risk human papillomaviruses: one type 16 and the other type 52. In the cytological examination results, women in the HSIL group were more likely to have HSIL/ASCH smears, while in the LSIL group, LSIL smears were more common compared to other groups of women with chronic cervicitis ($p < 0.05$). All women in the comparison group had only NIL smears ($p < 0.05$). In women with Ca in situ, ASCH and ASCUS smears were recorded. When comparing the results of cytological and histological examinations of cervical epithelium, the following results were obtained: in women in the LSIL group, 33.3% ($n=12$) of smears were consistent with LSIL.

The expected number of LSIL-type smears is at least 22 (women with CIN I), with 10 false-negative cytology results. In women with chronic cervicitis from the HSIL group ($n=21$), 6 false-negative cytology results were obtained. A total of 16 (28.0%) false-negative cytology results were obtained among women with SIL, which were not confirmed by histological examination.

Conclusion

1. Women with chronic cervicitis have a high incidence of squamous cell intraepithelial lesions of the cervix (71.3%): LSIL 45.0%, HSIL 26.3%. In women with chronic cervicitis and squamous cell intraepithelial lesions of the cervix, bacterial and viral associations predominate in the microflora of the cervicovaginal discharge.

2) Squamous cell intraepithelial lesions of the cervix in women with chronic cervicitis occur three times more often in the presence of obligate pathogenic infectious agents (*Chl. trachomatis* and/or *T. vaginalis*, *M. genitalium*) than in women infected with opportunistic flora. High-grade squamous intraepithelial lesions occur in more than 70% of women with chronic cervicitis coinfecting with high-risk HPV.

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