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Research Article

Proliferative Activity of the Epithelium of the Mucous Membrane of the Cervix of Postmenopausal Women

IV Reva[3,4], T Yamamoto[4], IA Odintsova[5], SN Nikolaenko[1,4], GV Abramyan[1,4], YI Pigolkin[6], SN Vaschenko[7], DP Puga[1,4], VG Kojukhar[8], VV Usov[1,4], EI Valkovitsch[8], IA Khramova[9] and GV Reva[2,3,4]*

[1]*Far Eastern Federal University, Vladivostok, Russia*

[2]*Engineering School, Vladivostok, Russia*

[3]*Biomedicine School, Vladivostok, Russia*

[4]*International Medical Education and Research Center (IMERC), Niigata, Japan*

[5]*S.M. Kirov Medical Military Academy, St. Petersburg, Lebedeva Street, Russia*

[6]*First Moscow State Medical University named after IM Sechenov, Moscow, Russia*

[7]*Medical Center «Asklepiy»; Vladivostok, Ostryakova Street, Moscow, Russia*

[8]*St. Petersburg State Pediatric Medical University, Litovskaya street, Russia*

[9]*Pacific State Medical University, Vladivostok, Ostryakova Street, Moscow, Russia*

***Corresponding Author:** GV Reva, Professor of the Department of Fundamental Medicine of School of Biomedicine; Professor of the Department of the Oil and Gas Extraction and Petroleum Chemistry of Engineering School, Russia.

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Abstract

Cervical cancer is diagnosed in 600,000 women aged 35 to 55 years every year around the world, and in a half of cases oncologic process is revealed at a late stage already. It is assumed that human papillomavirus infection (HPVI) increases the risk of dysplasia in 10 times. Some authors associate malignancy with other sexually transmitted diseases.

Immunohistochemical method of detecting the activity of Ki-67 gene, the presence of nuclear protein of which is irrefutable proof of cell entry into irreversible mitosis, is recognized as one of the most informative methods for diagnosis of early carcinogenesis of cervix. In recent years, many researchers questioned positive results and diagnostic significance of detection of high activity of the protein markers Ki-67. Inconsistency and contentious debates on these issues formed the basis for our study - researching of the diagnostic utility of detection of nuclear protein Ki-67 gene in predicting of tissue malignancy.

The obtained information shows the low diagnostic utility of data on proliferative activity in the diagnosis of carcinogenesis but gives an exhaustive view about the state of the regenerative potential of the cervical epithelium in physiological and reparative regeneration against microbial contamination. In the case of papillomavirus infection, the loss of cells' ability to restitution, as a disruption of the basilemma are more significant signs of early malignancy of the cervical tissue. High proliferative activity of the epithelium in comparison with the norm against the background of chlamydial infection correlates with the acute infection step, and the low corresponds to the chronic process.

Keywords: *Cervical Epithelium; Ki-67 Protein Gene; Proliferation; Carcinogenesis; Mitotic Activity; Menopause; Papilloma; Polyp; Papillomavirus; Malignization*

Urgency of the Research

Scientific research conducted in the direction of identifying criteria for preliminary diagnosis of oncological diseases is one of the primary objectives under the programme of fundamental researches held in the Russian Federation and throughout the world. High mortality, low survival rates of patients due to late diagnosis of cervical oncology produces a need to the development of new methods for detecting cancer [6]. According to statistics, this pathology is diagnosed in approximately 500,000 new cases every year, thus there are 14 - 16 cancer patients with cervical cancer per 100 000 population. The issue of the diagnostic significance of the proliferative activity of the epithelium in the diagnosis of cells and tissues malignancy is now controversial, since the main questions in the pathogenesis of tumors are still unresolved [8]. At this stage of researches the etiological key importance of the human papilloma virus (HPV), trichomoniasis, chlamydiosis and other infections in the cervix carcinogenesis is the subject of contentious debates. The issue of the origin of tumor cells is controversial, the malignization of cambial tissue cells and the oncological mutational transformation of the genome are open to question, since transformed cell is immediately came under to apoptosis. The advanced concept of cancer cells circulation in the blood hasn't yet been confirmed. Attempts to treat cancer patients with the injection of HSC programmed differentiation stem cells have been unsuccessful because there is undeveloped and unreasonable conceptual platform under them [9]. The significance of the Ki-67 gene activity in malignancy is now in question. Therefore, at this stage diagnosis and preventive measures, unfortunately, are purely empirical, and sometimes speculative, and they require getting the additional scientific facts. This was the basis for our choice of the scientific research direction.

The Goal of Research was to identify the activity of the Ki-67 gene in diagnosis of the mucous membrane of the cervix pathology in the zone of transition of stratified squamous epithelium into a single-layered cylindrical epithelium against the background of microbial infections in postmenopausal women.

Study Materials and Research Design

The research was carried out taking into account the provisions of the Declaration of Helsinki (2000) and with the permission of the Ethical Committee of the Far Eastern Federal University. Biopsy samples of the mucous membrane of the cervix and uterine body were obtained according to order of the Ministry of Health and Social Protection of the Russian Federation of 29.04.94 No. 82 "Of the procedure for performing post-mortem examinations" and according to nomenclature of clinical laboratory studies of the Ministry of Health of the Russian Federation (Order No. 64 of February 21, 2000). The material from postmenopausal women without any inflammatory processes in the mucous membrane of HSC was used as a control in the amount of 5. The material from women against the backgrounds of chlamydial [7], trichomonas [5] and papillomavirus [11] infections was studied in a comparative aspect. The biopsy material was fixed in accordance with the prescription for preparation for immunohistochemical studies straight after the withdrawal. We used classical histological methods of staining with hematoxylin and eosin for obtaining a general morphological pattern, as well as an immunohistochemical method for detecting the proliferation marker of cells - proliferating cell nuclear antigen Ki-67. The distribution of the clinical material is presented in table 1.

№	Control	Chlamydiae	Trichomoniasis	Papillomas
1	5	7	5	11
Total			28	

Table 1: *The distribution of the clinical material.*

Paraffin sections with a thickness of 5 μm were mounted on glasses pretreated for 5 minutes with 0,01% poly-lysine solution (Poly-l-Lyzine solution 0,1%, SigmaUSA) and dried in a thermostat at 56˚C for an hour. After the standard removing of paraffin procedure in toluene and alcohols, the sections were baked in a special solution (Target Retrieval Solution, DAKO, Denmark) in water bath at a temperature

of 95 - 97°C for 30 minutes for the reduction of the antigenic structure. The glasses were then were cooled to room temperature, washed 5 minutes with a 3% hydrogen peroxide solution in order to suppress endogenous peroxidase, and then washed in 3 changes of 0,02M phosphate buffer with pH 7,5 for 5 minutes for each. After that, primary antibodies to the Ki-67 protein (DAKO, Denmark) were applied, incubated for 30 minutes in a moist chamber in a thermostat at 37°C, washed in 3 changes of 0,02 M phosphate buffer pH 7,5 for 5 minutes for each. Horseradish peroxide-conjugated (Streptavidin Peroxidase Conjugated, DAKO, Denmark) was then applied. The intensity of staining was controlled using a microscope (incubated with DAB for 3 to 5 minutes). After the appearance of the brown staining of the nuclei, the sections were rinsed in distilled water for 5 minutes, finished a staining with haematoxylin, then encased in balsam. As a result of the treatment, the nuclei of proliferating cells are detected in the S-period, when the maximum of the Ki-67 gene protein synthesis correlating with the concentration of DNA is observed.

The material was analyzed with the Olympus-Bx82 microscope and the CDx82 digital camera with a firmware.

Research Results and Discussion

The main methods of diagnosis of changes in the cervix were examination in mirrors, simple and extended colposcopy, assessment of vaginal microbiocenosis, cytological examination of impression smears (so-called PAP-smears) and target biopsy followed by histological examination. Given that inflammation of exo- and endocervix can simulate a pattern of cellular atypia during a cytological study, all morphological studies were performed after the sanitation of the vagina. We noted that among women aged 46 years and older ectopia was observed in 7,3% of cases. Sometimes colposcopic pictures can be variegated and combined with a transition zone, but in ectopia of postmenopausal women they didn't have significant differences. It has been established that in the postmenopause the ectopia in the form of focal areas of the cylindrical epithelium was not detected in either case. Sometimes residual effects of ectopia in the form of a transformation zone (TZ) with open and closed glands were observed.

Against the ectopia polyps were found in 2,8% of cases, while in the group of women older than 45 years it was observes in 15% of cases. Polyps more frequently develop at the age of 40 - 50 years and recurring of polyps was observed in 18% of patients. Polyp is the proliferation of the mucous membrane of the cervical canal with involvement of the underlying fibrous tissue in this process. The reasons of the polyps' occurrence in the examined patients were associated with a disorder of hormonal and immunogenic homeostasis, and inflammatory processes. Polyps in our observations were less often covered with a cylindrical epithelium, while they had a bright red acinous surface. More often, the polyps were covered with stratified squamous epithelium, in this case they were pink and smooth.

In our researches, when polyps were detected on the uterine cervix, it was noted that this focal proliferation of the stratified squamous epithelium along with the underlying connective tissue with the phenomena of cornification. Externally the papilloma is a pink or whitish verrucose formation. In most cases, the base of the papilloma was broad, less often - in the form of a thin pedicles, and sometimes an external growth was noted (Figures 1 and 2).

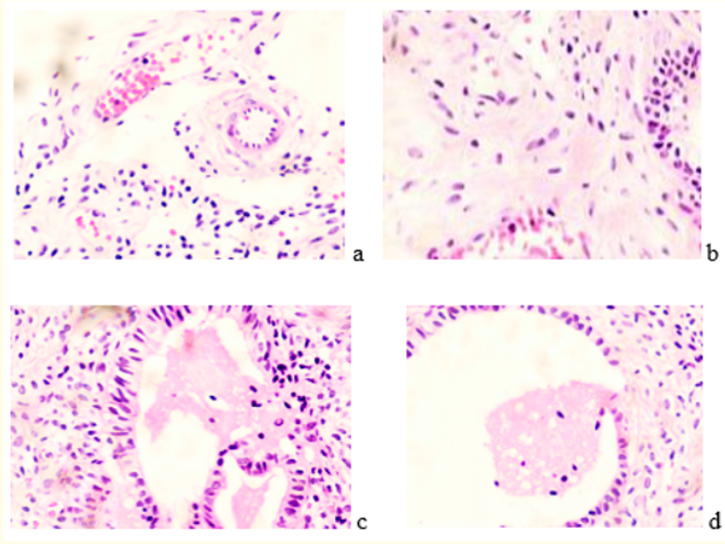

Figure 1: *Cervical mucosa of postmenopausal women. The polyp of the cervical wall of 49 years old woman is in the zone of transition of stratified squamous epithelium into a single-layered cylindrical epithelium. Microphoto. Staining with haematoxylin and eosin. Magnificationx200.*

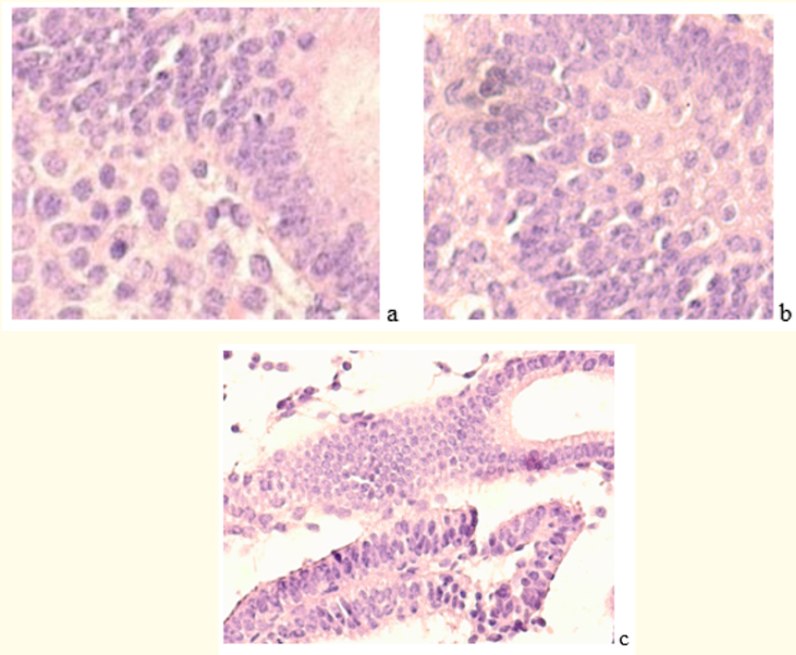

Figure 2: *Cervical mucosa of postmenopausal women. a, b) Woman, 41 years old. Polyp. Diagnosis for malignancy prediction. The polyp was removed. c) Woman, 63 years old. Polyp. Diagnosis for malignancy prediction. The polyp was removed. Staining with haematoxylin and eosin. Microphoto. Magnification: a, b - x400; c - x200.*

Disorder in the structure of the basal and free plane of the surface epithelium and hyperaemia of the vessels of the proper mucous plate were noted. The basilemma of the epithelium was not identified, leucocytic infiltration was observed not only in the proper mucous plate but also in the lumina of the glands.

Analysis of the proliferative activity of the cervix mucosal epithelium made it possible to establish that, despite the duration of postmenopause, weak proliferative phenomena in stratified squamous and cylindrical epithelium of the cervix can be observed during this period and, respectively, in the first year of postmenopause, proliferative types of smears amount 75% (Figure 3).

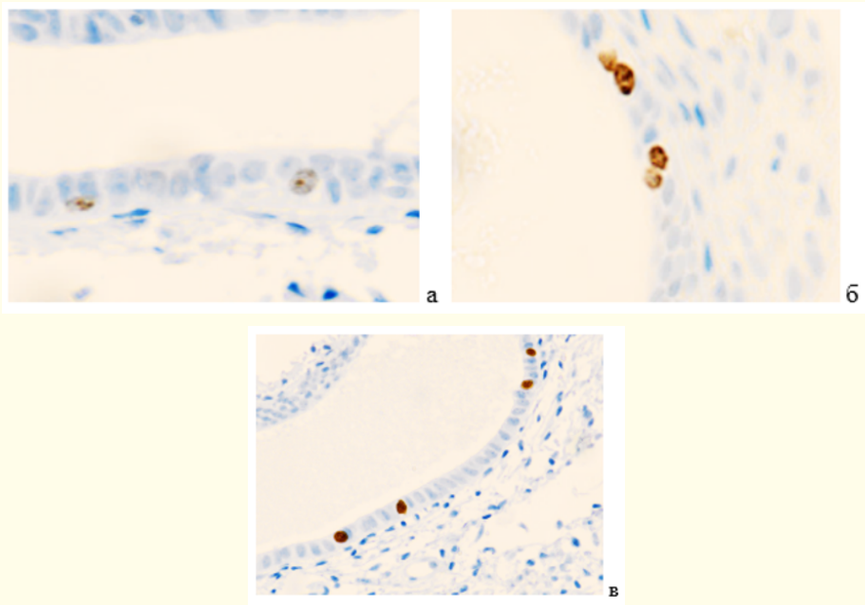

Figure 3: *Cervical mucosa of postmenopausal women. Localization of the protein of the Ki67 gene in the structures of the cervical mucosa is normal against the background of postmenopause. Microphoto. Immune histochemistry and finishing staining with haematoxylin. a, b, c - normal. Magnification: a, b – x20; c – x200.*

In the presence of polyps and inflammatory processes due to chlamydial and trichomonous infections in the cervical mucosa the proliferative activity not only increases in the epithelial plate, but also takes place in its own plate (Figures 4 and 5).

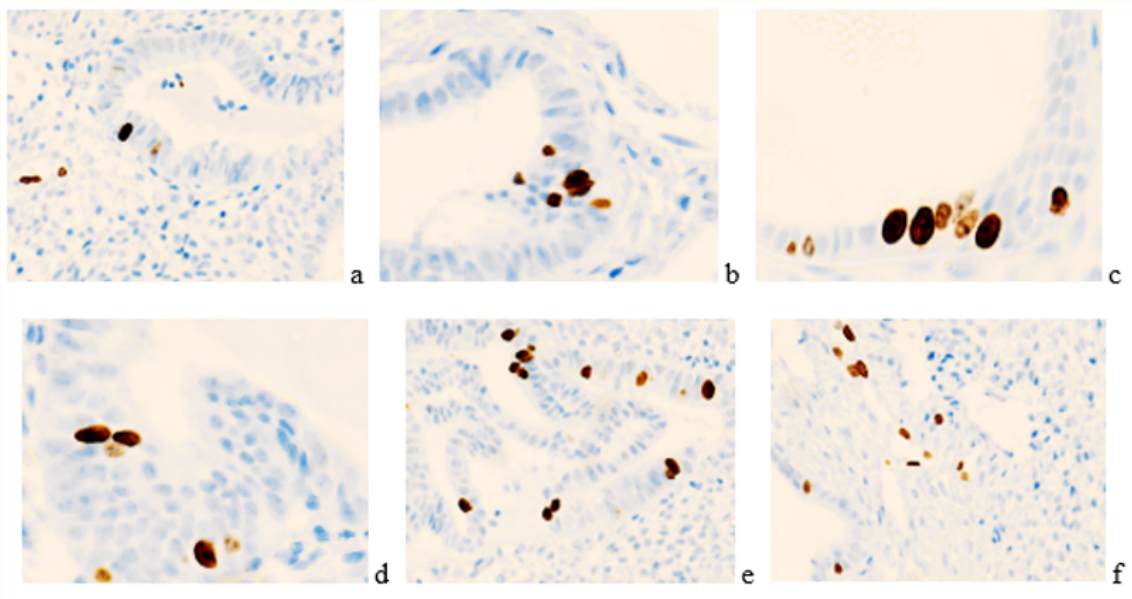

Figure 4: *Cervical mucosa of postmenopausal women. Localization of the protein of the Ki67 gene in the structures of the cervical mucosa in the presence of polyps. Microphoto. Immune histochemistry and finishing staining with haematoxylin. Magnification: a, e, f - x200; b, c, d - x400.*

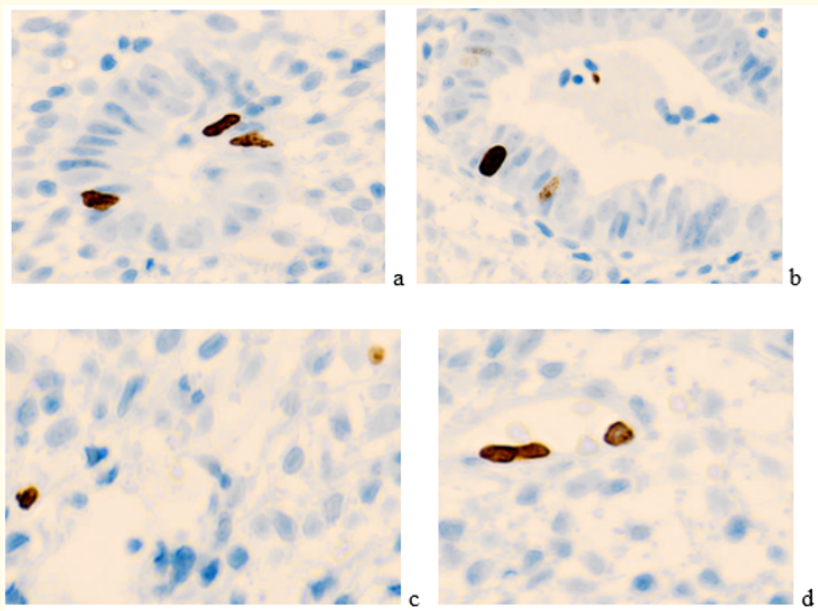

Figure 5: *Cervical mucosa of postmenopausal women. Localization of the protein of the Ki67 gene in the structures of the cervical mucosa in the presence of chlamydial (a, b) and trichomonous (c, d) infections. Micro photo. Immune histochemistry and finishing staining with haematoxylin. Magnification: a, e, f - x200; b, c, d - x400.*

We noted disorders in the structure of the epithelial plates and lamina propria of the mucous membrane, the disruption of the basilemma, while the daughter cells bloom to the surface of the mucous membrane.

It was noted that in case of chlamydial infection the atrophy of the cylindrical epithelium is most franked with the lowest proliferative activity of the epithelium.

Information on the proliferative activity of the Ki67 gene in the structures of the cervical mucosa is presented in table 2.

№	Control	Papillomas	Trichomoniasis	Chlamydiae
1	3,2 ± 0,12	8,4 ± 0,23	2,6 ± 0,11	2,4 ± 0,09

Table 2: *Proliferative activity of the Ki67 gene in health and in disease of the cervix in postmenopausal women. *P < 0,05.*

Lower proliferative activity in the structure of the epithelial plate in the case of chlamydial infection is in accordance with literature data on inflammatory processes and erosions that can't be treated against this pathology, and also indicates the presence of a chronic infection. In case of long-lasting contamination of women with HPV apoptosis was observed (Figure 6).

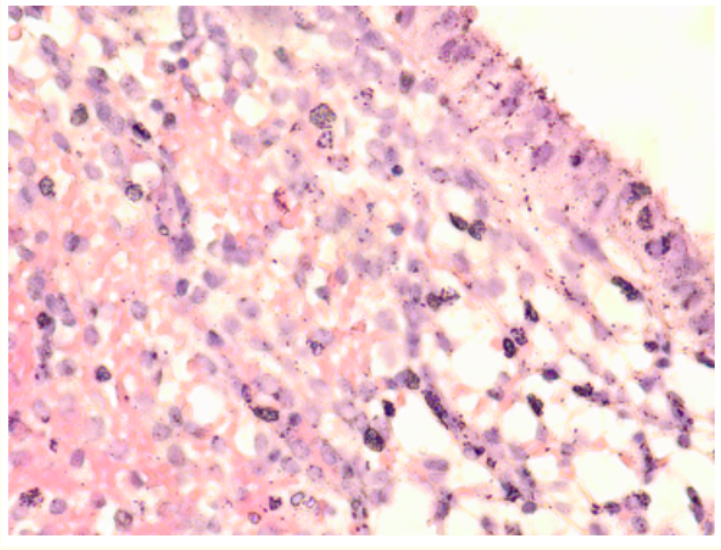

Figure 6: *Cervical mucosa of woman, 63 years old. Polyp. Diagnosis for Malignancy Prediction. The polyp was removed. Apoptosis, microbial contamination with chlamydiae. Staining with haematoxylin and eosin. Microphoto. Magnification: a, b - x400; c, d - x200.*

Against the age-related estrogen deficiency and overlaying of chlamydial and other infections there are more franked morphological changes associated with epithelial damage, presented as a type of atrophic colitis (vaginitis) and nonspecific cervicitis. At the same time, there are dystrophic changes in the underlying stroma, associated with worsening of trophicity, decreasing of microcirculation of blood flow and processes of transudation in stroma and all layers in the mucous membranes of the reproductive tract of postmenopausal women.

Conclusion Report

There are actually no targeted strategies for predicting the fate of cancer of the women's reproductive system now [1,5,16]. Sui M, Pei Y, Li D, Li Q, Zhu P, Xu T, Cui M (2016) as well as other authors note that the delay in diagnosis and treatment can lead to irreversible damage and severe disease [15]. The authors noted that there were rare cases of cervical adenocarcinoma, which is difficult to diagnose because of deep location, endogenous growth, deceptively nonmalignant appearing of the tumor cells and lack of communication with the human papilloma virus (HPV). Liang SN, Huang YJ, Liu LL., *et al.* (2015) consider that the expression of Ki67 gene is closely associated with the occurrence and development of cervical carcinoma, noting that there is a positive correlation between Ki67 gene and malignancy, and that it can serve as a biomarker for cervical cancer [11].

Kanthiya K, Khunnarong J, Tangjitgamol S., *et al.* (2016), analyzed the 40-year base of literature data in the evaluation of Ki67 expression in cases of cervical intraepithelial neoplasia (CIN) and cancer, in contrast to the majority of authors who considered Ki67 expression in 100% of all invasive carcinomas. The analysis results showed 75,4% of the cases of CIN 2 - 3, 22,6% of the cases of CIN1, and 11,3% of the cases of neoplasia, and obtained a direct association of Ki67 gene activity with the severity of cervical disease especially exhibiting high sensitivity and specificity for CIN2. There was a lack of protein detection in 6 and 7 cases of CIN1 and CIN2, respectively [10].

According to Zhao J, Guo Z, Wang Q, Si T., *et al.* (2017), the cervical cancer mortality is associated with genotypes of papillomaviruses 16, 18, 58 and 52 [17], but other authors [7,12,13] consider cytological studies to be more reliable.

There were very few large-scale studies dedicated to contamination by the human papillomavirus virus and determination of the dominant type of virus for women in our country. Programs existing at the current stage do not allow assessing the scale of the defeat of women by the human papillomavirus and other infections that occur without vivid clinical symptoms in our country. Nevertheless, the existence of more than 200 strains of human papillomavirus, spontaneous recovery in 98% of cases does not support the advisability of vaccination. The data available at the time indicate the coincidence of prevalence ratios of types 16 and 18 of papillomavirus infection in the Russian Federation and in European countries [2]. High oncogenic types of viruses amount 58% and that fact can alert and explains the high incidence of cervical cancer. The expansion of diagnostic resources, including monitoring of proliferative activity based on analysis and comparative characteristics of regenerative potential indexes in the cervical mucosa with the health model for the corresponding age group, is the most promising in the development of strategies for the prevention, treatment and rehabilitation of postmenopausal women.

As in the works of Prilepskaya VN (2008), we observed that regardless of the duration of postmenopause weak proliferative phenomena could be in stratified squamous and cylindrical epithelium of the cervix during this period [3].

Our results are consistent with the results of Calil LN, Igansi CN, Meurer L, Edelweiss MI, Bozzetti MC (2011), we also found a statistically significant association between cervical mucosa diseases caused by HPVI, Trichomonas, Chlamydia trachomatis and Ki67 gene activity [4].

At the same time, we noted that against the background of a chronic course of infections, the activity of Ki67 gene is statistically significantly reduced which may be due to damage of cambial cells and depletion of the regenerative potential.

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