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THE ROLE OF BIOCHEMICAL MARKERS IN SCREENING OF CERVICAL EPITHELIAL PATHOLOGY WITH DETERMINATION OF P16INK4A PROTEIN EXPRESSION

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Abstract
This article describes the role of biochemical markers in the pathology of the cervical epithelium. In research 10716 women aged 25-65 years were examined (mean age 43.28±3.82 years). And in the course of the study, it was found that the expression of the P16INK4A protein by cervical epithelial cells increases the risk of epithelial metaplasia of any degree by 8.86 times, high-grade epithelial metaplasia - by 692 times, RMS by 48 times.

Keywords Cervical cancer, early diagnosis, biochemical markers, P16INKK4A.

INTRODUCTION

More than 300,000 women die from cervical cancer each year. About 90% of new cases and deaths in 2020 occurred in low- and middle-income countries. In Uzbekistan, cervical cancer is the second most common cancer among women of all ages and the second most common cause of death among women of childbearing age after breast cancer [5,7]. Most of these deaths are due to late diagnosis. In this regard, the most pressing issues are to improve the detection, diagnosis and prevention of precancerous and cancerous diseases of the cervix [3].

Currently, the most relevant research in the world practice is to improve the early diagnosis, treatment and prevention of cervical cancer, the diagnostic, prognostic and immunological role of the expression of various proteins is being studied, which can provide additional opportunities for establishing diagnostic and prognostic biomarkers, as well as targets for preventive action. [8] Gene Expression Omnibus (GEO) datasets are being explored using comprehensive bioinformatics tools and identifying biomarkers associated with cancer. National models for the early diagnosis of precancerous lesions of the cervix are being developed using large-scale screening datasets, as well as tele-education and tele-mentoring programs for cervical cancer prevention [1].

In world practice, large-scale work on social protection of the population and improvement of the healthcare system is currently ongoing. In this direction, in particular, in improving the results of early diagnosis and prevention of precancerous and cancerous diseases of the cervix, positive results have been achieved [5]. At the same time, to improve the provided diagnostic and preventive care, evidence-based results are required to optimize the screening of precancerous and cancerous diseases of the cervix. The development strategy of New Uzbekistan for 2022-2026 in seven priority areas includes tasks to improve the quality of the provision of qualified medical services to the

population. The implementation of these tasks, including the improvement of the results of treatment and prevention of cervical cancer through a new innovative approach to early diagnosis and prognosis, is one of the topical areas[2,5].

MATERIALS AND METHODS

In the course of this study during 2020-2021 10,716 women aged 25-65 years (mean age - 43.28±3.82 years) were examined, who did not present complaints, indicating gynecological pathology and did not have a history of erosive lesions of the cervix. All examined women underwent a PCR test to detect HPV DTCs in the cells of the cervical mucosa. Cells were obtained by taking with a special brush. As a result, groups of HPV-positive (HPV+) and HPV-negative (HPV-) women were identified. Also, all the subjects underwent an examination of the cervix in gynecological mirrors to visually determine the state of the mucosal epithelium and cytological examination by the Papanicolaou method. During the examination, all examined women were divided into a group with unchanged cervical mucosa (PAP-) and a group with erosive changes in the mucosa.

All women included in the HPV+ and PAP+ groups underwent colposcopy and biopsy of the cervical mucosa, as well as the determination of the SCC tumor marker and the determination of P16INK4A protein expression.

In case of detection of LSIL, electrocoagulation of the affected area of the cervix mucosa was performed, HSIL - electroconization, carcinomas - hysterectomy.

During the study, the number of women with indications for various treatment options for cervical pathology was recorded, depending on the algorithm of diagnostic tactics.

At the first stage today is a cytological examination of the cervix, thanks to which malignant processes can be detected in the early stages. The cytological sampling is taken from three sites, from the vagina, at the site of the transition of the stratified squamous epithelium to the glandular one, from the cervical canal.

A disposable instrument is used to take the material, which includes a spatula, brush and glass slides. A fence is taken after the menstrual cycle, after careful processing and cleaning of cervical mucus, by lightly scraping with an Eyre spatula. The brush is also carefully inserted and a swab is taken from the canal in a clockwise circular motion.

Cytological studies with a parvil approach, atypical cells can be detected if there is no inflammatory process, when there is a microbial flora. Therefore, to start the study, it is necessary to sanitize the vagina and re-take the material for cytological examination.

Expression of the p16INK4a protein on the surface of epithelial cells is determined during immunocytochemical studies.This protein blocks the stimulation of cell division by inhibiting cyclin dependent kinase. The mechanism of mitosis inhibition by the p16INK4A protein is mediated by the retinoblastoma protein (pRb). Under conditions of decreased activity of cyclin dependent kinase, pRb phosphorylation stops, pRb binds and inactivates the E2F transcription factor, blocking its mitotic activity. HPV DNA in the host genome triggers the expression of the E6 and E7 oncogenes, which in turn bind pRb s, disrupt E2F inhibition, and promote uncontrolled mitotic activity. Interestingly, a negative p16INK4A test in HPV-positive women is associated with a low risk of precancerous cervical lesions.

An immunoassay that detects p16INK4a is another potential option for distinguishing patients at high risk of cervical cancer from HPV-positive women. Analysis of randomized data shows that if all HPV-positive women with p16INK4a are referred for colonoscopy, the need for colonoscopy is comparable to the need for referral during cytology screening, with a 53% increase in CIN2+ detection and higher. A more recent study using p16INK4a/Ki-67 dual testing (CINtec plus, Ventana) showed that this technique was more sensitive than cytology in detecting CIN2+ and more in HPV positive women. This provides a longer interval for HPV+ and p16/ki67- before

referral for colposcopy. Further research is needed to confirm the role of p16/ki67 immunoassay as a key test in HPV positive patients. HPV+ in combination with a positive p16ink4a test when referred for colposcopy showed a sensitivity of 97.8% in terms of CIN2+, while HPV-/P16in4A- was associated with a 3-year risk of developing CIN3+ - 0.2%.

RESULTS AND DISCUSSION

The P16INK4A protein was determined by immunohistochemistry in the cells of the cervical mucosa. Testing was conducted in women positive for HPV and cytological test (3108 people). Protein P16INK4A was determined in 398 women (12.81%). Most often, the protein was found in women aged 51-60 years (27.14% in the group of 51-55 years and 24.14% in the group of 56-60 years), least often in the group of 25-30 years (4.47%) and 31-35 years old (7.55%) (Fig. 1).

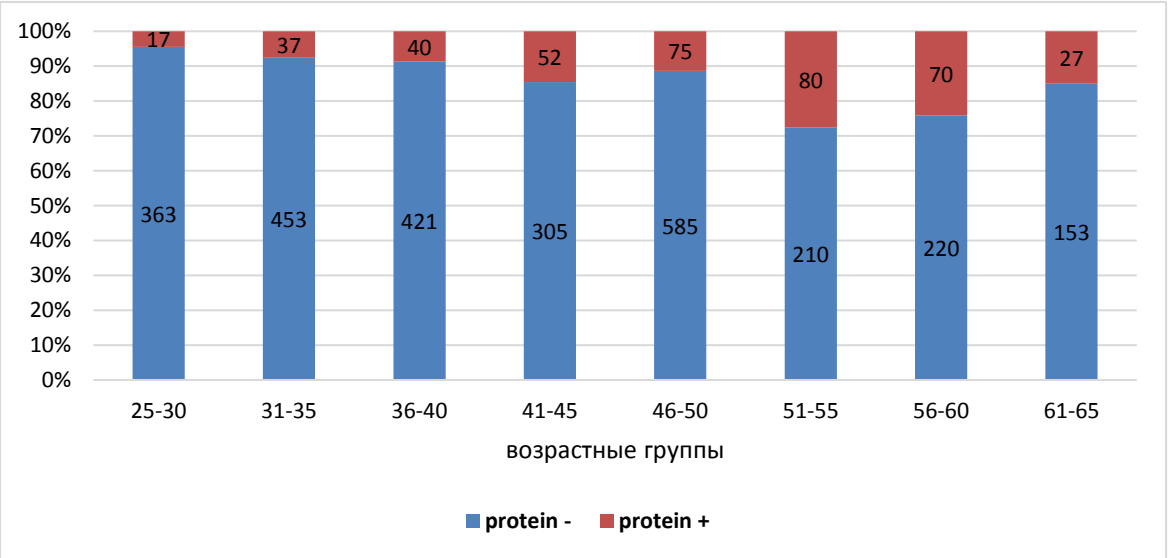

Fig. 1 Frequency of detection of P16INK4A expression in women with indications for colposcopy depending on age

Identification of groups according to the type of pathological changes in the CC epithelium (Table 1) showed that in women in whom no pathology was detected during colposcopy and or changes in ASCUS were detected, the expression of the P16INK4A protein was not determined. In women with LSIL class metaplasia, P16INK4A expression was observed in 194 out of 498 women (38.96%), while in women with high-risk metaplasia (HSIL) - in 195 women out of 197 (98.98%), and in women with diagnosed cervical cancer - in 100% of cases (7 out of 7).

Table 1

The frequency of detection of P16INK4A protein expression by cervical epithelium in women with indications for colposcopy of different ages and different types of epithelial metaplasia

Age grops	All pathology options	ACSUS	LSIL	HSIL	c-r
25-30 y.o	17 83	0 43	9 32	7 8	0 0
31-35 y.o	37 191	0 86	14 82	22 23	0 0
36-40 y.o	40 167	0 97	22 52	17 17	1 1
41-45 y.o	52 146	0 74	31 51	21 21	0 0
46-50 y.o	75 150	0 81	55 49	18 18	2 2
51-55 y.o	80 277	0 141	36 92	43 43	1 1
56-60 y.o	70 279	0 116	15 108	53 53	2 2
61-65 y.o	27 92	0 45	12 32	14 14	1 1
Total	398 1385	0 683	194 498	195 197	7 7

Note: (the numerator is the number of positive results, the denominator is the number of patients in the age group).

In general, in the examined women with pathological changes in the epithelium of the cervix, detected during colposcopy, the P16INK4A protein was expressed in 398 out of 1385 people (28.74%). Since the detection rate increased with the degree of metaplasia, this indicator can be used as a marker of high-risk epithelial metaplasia.

Among HPV-positive women with a positive

cytology test, detection of P16INK4A expression by cervical epithelial cells is associated with a 48-fold increased risk of cervical cancer, a 692-fold increased risk of high-grade epithelial metaplasia (HSIL+carcinoma), and an increased risk of any grade of epithelial metaplasia (LSIL). +HSIL+carcinoma) by 8.86 times (Table 2).

Table 2

The relative risk of developing cervical cancer, epithelial metaplasia of various degrees in women, depending on the expression of p16INK4a by epithelial cells of the cervical mucosa.

Pathology	P16INK4A+	P16INK4A-	χ^2	OP
Total	396	2712		
Cervical cancer	7 (1,77%)	0	42,54, p<0,001	48
HSIL+CC	202 (51,01%)	2 (0,07%)	1456,46, p<0,001	692
LSIL+HSIL+CC	396 (100%)	306 (11,28%)	1553,34, p<0,001	8,86

Thus, the study showed that the expression of P16INK4A by cervical epithelial cells can be included in the cervical cancer screening program as a marker of high-risk epithelial metaplasia.

CONCLUSIONS

Detection of P16INK4A protein expression by CMM epithelial cells increases the risk of epithelial metaplasia of any degree by 8.86 times, high-grade epithelial metaplasia by 692 times, and RMS by 48 times. The determination of the SCC protein in the serum of women with a positive HPV test or cytological examination has no diagnostic value in terms of the risk of cervical cancer.

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