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IMMUNOHISTOCHEMICAL PREDICTORS OF THE COURSE AND OUTCOME OF TREATMENT IN CERVICAL CANCER

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Summary

Cervical cancer currently ranks third in oncogynecological morbidity among malignant diseases of the female reproductive system in the Republic of Uzbekistan. According to available literature data, squamous cell carcinoma accounts for up to 77+3% of cases, while adenocarcinoma constitutes 11% and sarcoma 1%.

Purpose of the study: *to investigate immunohistochemical predictors influencing the prognosis of clinical course and outcome of cervical cancer treatment.*

Material and methods of research: *We analyzed immunohistochemical (IHC) results from 40 cervical cancer patients treated at the Samarkand Branch of the Republican Specialized Scientific-Practical Medical Center of Oncology and Radiology during 2020-2023. The cohort included 20 patients with disease recurrence after complex treatment and 20 recurrence-free patients. All studied cases were histologically confirmed as squamous cell carcinoma of the cervix (T1b-3aNxM0).*

Results. *Immunohistochemical evaluation of Ki67, VEGFR, and CD34 markers proved most valuable for treatment planning and disease prognosis. The study demonstrates characteristic changes in Ki67, VEGFR, and CD34 expression patterns according to tumor grade (G-classification).*

Conclusions. *Comprehensive histopathological and immunohistochemical analysis of cervical cancer represents a technically demanding and time-consuming procedure. However, its diagnostic accuracy enables optimal treatment selection, therapy response monitoring, and improved disease outcome prediction.*

Keywords: *Cervical Cancer, Immunohistochemical Study; Degree of Proliferative Activity, Recurrence; Chemotherapy.*

Introduction

Cervical cancer remains one of the main causes of morbidity and mortality among women in Uzbekistan [1] and other CIS countries, including Belarus [2, 3], Russia [4, 5] and Kazakhstan [6, 7].

According to World Health Organization data, cervical cancer incidence in Uzbekistan is approximately 5.5 per 100,000 women [8,9], compared to 10.5 per 100,000 in Russia [10,11], 5.5 per 100,000 in Kyrgyzstan [12], and 7 per 100,000 in Kazakhstan [13]. Belarus reports an incidence of 8 per 100,000 women with a decreasing trend (–2.7%) during 2010-2019 [14]. Within oncogynecological malignancies, cervical cancer ranks third in prevalence, with squamous cell carcinoma accounting for up to 77+3% of cases, adenocarcinoma for 11%, and sarcoma for 1%. Recent epidemiological studies demonstrate increasing diagnosis among socially active women aged up to 65+3 years [15, 16].

Despite early detection and comprehensive treatment, some cases experience rapid recurrence leading to mortality within 2 years, while other patients diagnosed at advanced stages (2b-3) achieve 5-year survival following combination therapy [17].

The clinical implementation of immunohistochemical predictors could significantly enhance cervical cancer treatment efficacy by enabling precise tumor molecular profiling and personalized therapeutic approaches [18], particularly relevant for CIS countries requiring context-specific treatment optimization [19]. These biomarkers may not only improve care quality but also establish new diagnostic and treatment standards [20].

Epidemiological data reveal rising incidence and mortality rates for cervical cancer in the Russian Federation and other regions during recent decades [21-25]. Current literature positions cervical cancer among the most prevalent malignancies in Belarus and Russia, ranking fourth worldwide among female cancers and seventh in overall cancer statistics [26-28].

Notably, increasing breast cancer incidence among young women has heightened the urgency for improved diagnostic and prognostic tools. Cervical carcinogenesis involves complex multifactorial mechanisms requiring evaluation through various immunohistochemical markers. Recent studies assessing p53, Ki67, cyclin D1, and CD45 protein expression aim to determine squamous cell carcinoma biological potential and develop early detection methods. However, data remain limited regarding local immunity's role in evaluating invasive and metastatic potential [3,29,30].

Immunohistochemical markers including p53, Ki67, cyclin D1, and CD45 are utilized internationally to predict treatment outcomes, achieving five-year survival rates ranging from 12% to 75% [31].

Immunohistochemical examination (IHC) represents a morphological technique that identifies tissue antigens, enhancing diagnostic specificity when combined with conventional histopathology. Globally recognized as the gold standard [32,33], IHC complements morphological evaluation for precise cancer diagnosis and treatment optimization.

A current research priority involves investigating early breast cancer recurrence among socially active women,

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driving interest in biomarkers that could enhance standard cytohistopathological assessment for malignant cell detection.

IHC methodology enables antigen identification in biological tissues, providing critical adjunctive data to morphological examination. Contemporary oncology practice employs IHC for more accurate tumor malignancy grading. This technique, when combined with traditional histopathology, improves diagnostic accuracy and therapeutic outcomes, maintaining its status as the global diagnostic benchmark.

Purpose of the study. To evaluate immunohistochemical predictors influencing cervical cancer prognosis and treatment outcomes.

Research materials and methods

We analyzed IHC results from 40 cervical cancer patients (20 with recurrence post-combination therapy and 20 recurrence-free cases) treated at the Samarkand Branch of the Republican Specialized Scientific-Practical Medical Center of Oncology and Radiology (RNPMCOiR) during 2020-2023. The study cohort comprised squamous cell carcinoma cases (T1b-3aNxM0). IHC analysis was performed at the Pathomorphology Department of the Russian Academy of Medical Sciences using the Bond Leica Australia immunohistoprocessor. Nuclear staining via the ALL RED method assessed Ki67, VEGFR, and CD34 expression, with results categorized by low, medium, or high proliferative activity.

Results

Comprehensive evaluation followed cervical cancer diagnostic standards, including: Clinical history assessment, reproductive function analysis, gynecological diseases, gynecological examination (documenting cervical tumor characteristics), examination of the uterine body (size, deviation, mobility, soreness), condition of the uterine appendages and parametric cells, smear and biopsy of the tumor from the cervix and cervical canal for oncocytology and histological examination), colposcopy, ECG and cardiologist examination, Chest X-ray, rectoscopy, cystoscopy, excretory urography and virtual colonoscopy MSCT, pelvic MRI, PET-CT, IHC examination. IHC utilized archival tissue blocks with Ki67, VEGFR, and CD34 antigen detection.

The function of these antibodies is as follows: Protein Ki-67, a tumor antigen (also known as MKI67), serves as a cellular marker of proliferation and is an excellent indicator for determining the growth fraction of a given cell population. The percentage of Ki-67-positive tumor cells (Ki-67 labeling index) is frequently correlated with the clinical course of cancer.

Vascular endothelial growth factor (VEGF) is a signaling protein produced by cells to stimulate vasculogenesis (formation of the embryonic circulatory system) and angiogenesis (growth of new blood vessels from pre-existing vasculature).

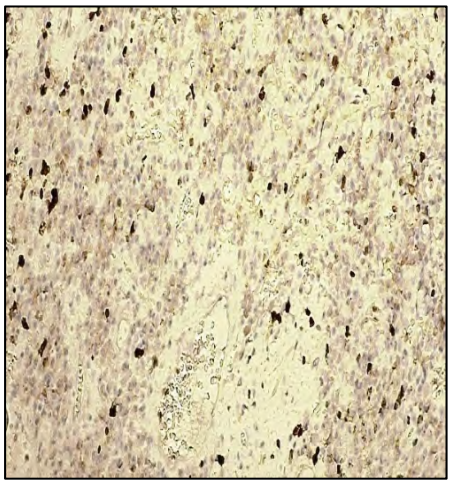
CD34 is a transmembrane glycoprotein involved in early hematopoiesis. It exhibits high sensitivity to vascular tumors and is expressed in both blood and lymphatic vessel endothelium. It may be utilized for early detection of lymphogenic tumor metastasis.

Table 1

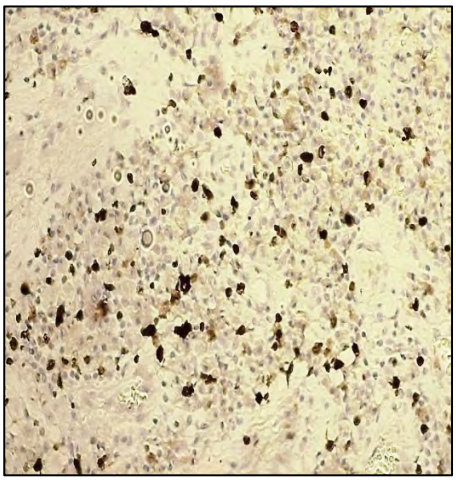
The degree of proliferative activity of Ki 67 in squamous cell carcinoma with a G-graded tumor.

G tumor gradation	G1	G2	G3
The degree of proliferative activity	Low proliferative activity –5 (25%)	10-20% average proliferative activity –5 (25%)	>20% high proliferative activity –10 (50%)

In a study of 20 patients with squamous cell carcinoma of the cervix without recurrence, the assessment of proliferative activity by Ki-67 expression according to tumor grade yielded the following results: in G1 squamous cell carcinoma of the cervix, low proliferative activity was detected in 5 (25%) patients; in G2, moderate proliferative activity (10-20%) was observed in 5 (25%) patients; and in G3, high proliferative activity (>20%) was noted in 10 (50%) patients. G3 squamous cell carcinoma of the cervix carries a high risk of disease recurrence. The microscopic findings are presented in Figure 1.

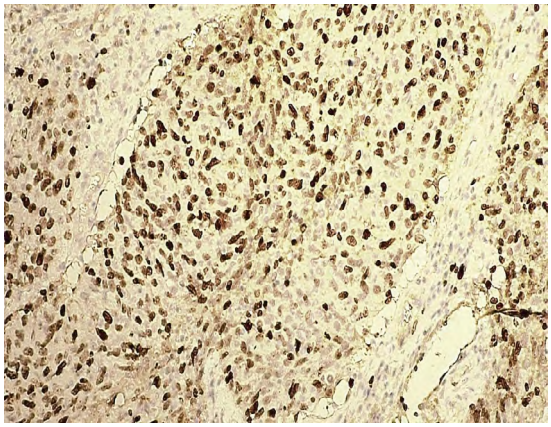


Squamous cell carcinoma G-1 of the cervix Ki67 high positive reaction of IHC-Comp chromagen. № 10. col40



Squamous cell carcinoma G-2 of the cervix moderate positive IHC reaction – Comp chromagen. № 10. col40

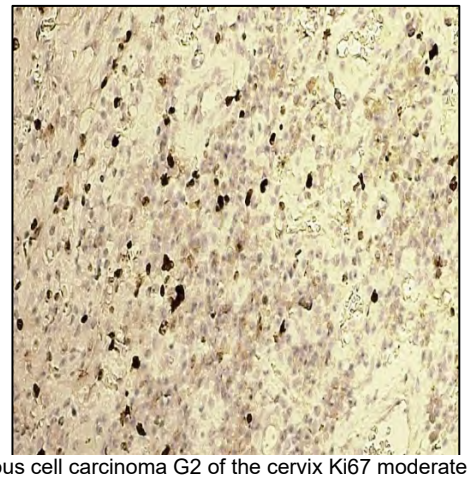
Fig. 1. IHC study of Ki67 in patients with cervical cancer without recurrence of the disease.



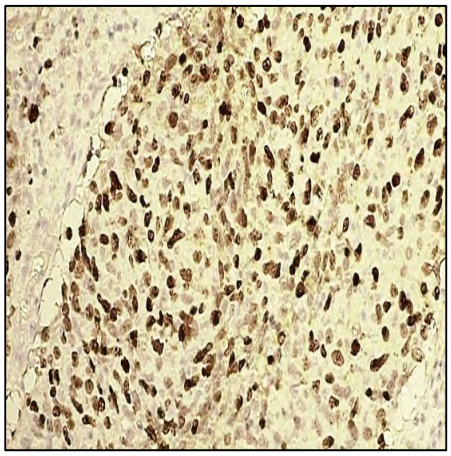
Squamous cell carcinoma of the cervix. G-3. Ki67 low positive IHC response –Comp chromagen. № 10. col40.

Fig. 2. IHC study of Ki67 in patients with cervical cancer without recurrence of the disease.

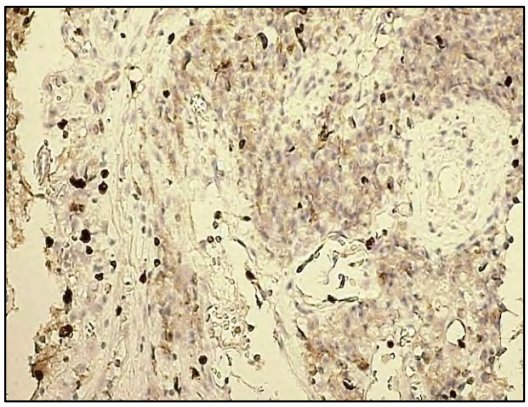
Microscopic examination revealed Ki-67-positive hyperplasia, squamous epithelial cell atypia, nuclear polymorphism, and numerous atypical mitoses. The nuclei of malignant cells exhibited dark brown staining. Cervical cancers with a high proportion of dividing cells – and consequently a high Ki-67 index – typically respond well to chemotherapy, as cytotoxic agents target cells during active division. Thus, a higher Ki-67 index correlates with greater treatment efficacy. Determination of the Ki-67 index aids in selecting the most appropriate therapeutic approach for individual cases.



Squamous cell carcinoma G2 of the cervix Ki67 moderate positive IHC response – Comp chromagen. № 10. col40. RELAPSE



Squamous cell carcinoma G3 of the cervix low positive IHC response – Comp chromagen. № 10. col40. RELAPSE



Squamous cell carcinoma of the cervix. G-1. Relapse. Ki67 low positive IHC response – – Comp chromagen. № 10. col40.

Fig. 3. IHC study of Ki67 in patients with recurrent cervical cancer.

A lower Ki-67 index correlates with poorer tumor response to chemotherapy (and vice versa). In low-grade breast cancer, reduced expression of smooth muscle actin was observed in both the tumor stroma and blood vessel walls, indicating a high metastatic potential.

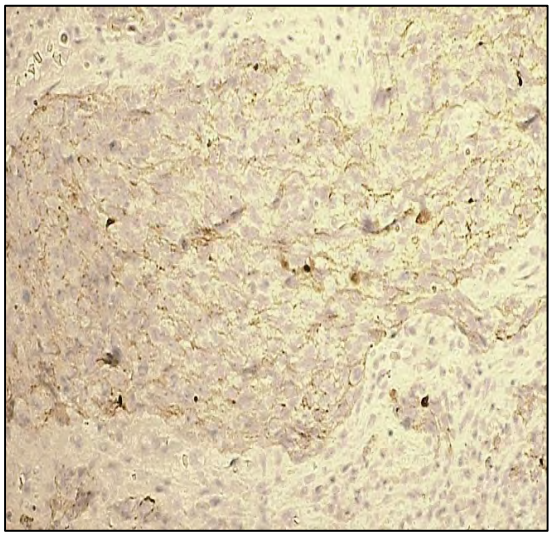
Evaluation of vascular endothelial growth factor (VEGF) revealed the following pathological changes. In the study group of 20 patients, 2 (10%) exhibited a mildly positive response, 7 (35%) a moderately positive response, and 11 (55%) a strongly positive response to the VEGF tumor marker (Table 2).

Table 2

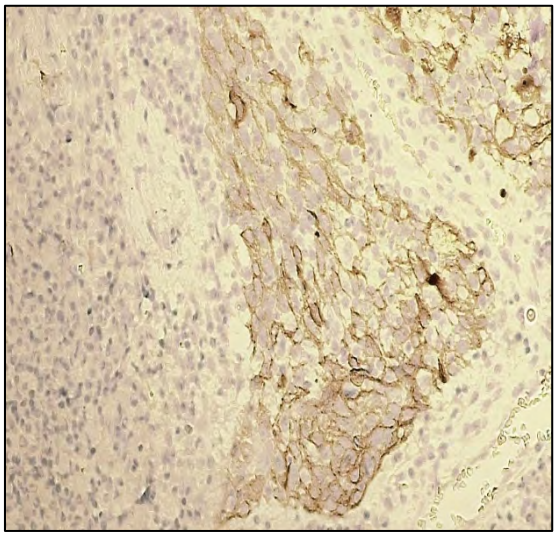
The degree of proliferative activity of the VEGF reagent in squamous cell carcinoma.

G tumor gradation	G1	G2	G3
Degree of activity	<30% low proliferative activity –2 (10%)	30-60% moderate proliferative activity –7 (35%)	>60% high proliferative activity –11 (55%)

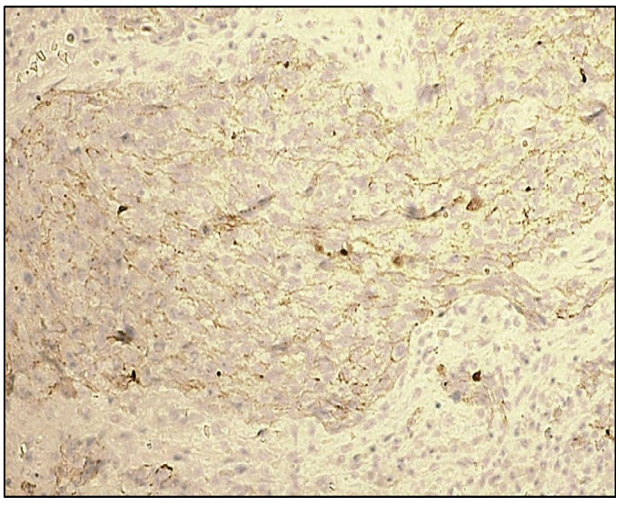
Microscopic examination of specimens from the 20 selected patients demonstrated squamous epithelial hyperplasia with atypical epithelial cells displaying nuclear polymorphism, arranged in irregular layers, alongside numerous pathological mitoses. Dark brown-stained vascular structures of varying sizes were densely distributed, with a frequency of 30-40 per field of view (Fig. 4).



Squamous cell carcinoma G-1 of the cervix low positive reaction of the VEGFR IHC reagent –Comp chromagen. № 10. col40



Squamous cell carcinoma G-2 of the cervix low positive reaction of the VEGFR IHC reagent –Comp chromagen. № 10. col40



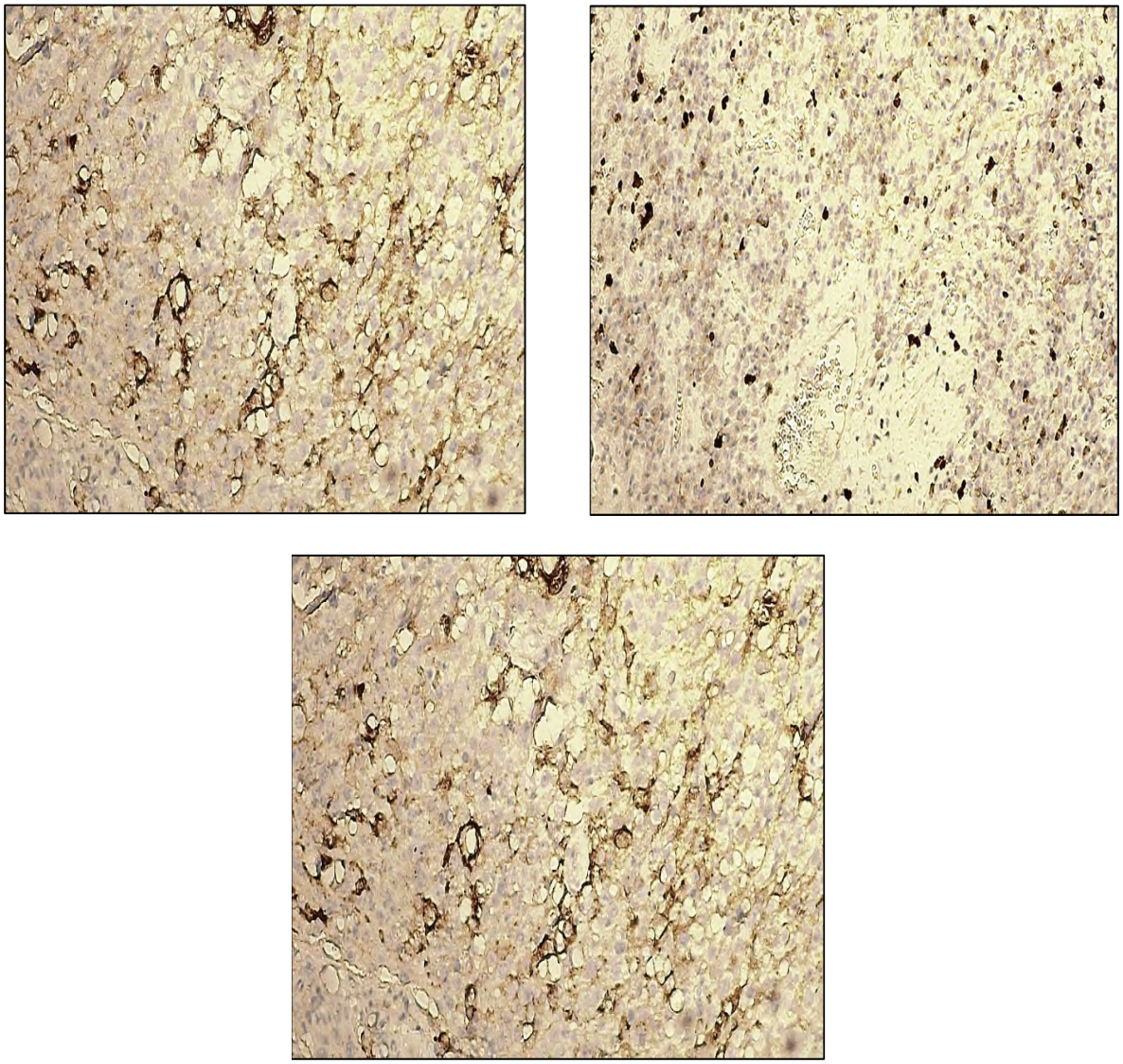
Squamous cell carcinoma of the cervix. G-3. Without relapse. VEGFR low positive IHC response – Comp chromagen. № 10. col40.

Fig. 4. The degree of proliferative activity of the VEGF reagent in squamous cell carcinoma of cervix.

Histopathological analysis revealed squamous epithelial hyperplasia with atypical epithelial cells organized in irregular layers, forming atypical malignant tumor cells exhibiting polymorphism and frequent pathological mitoses. The nuclei of the tumor cells were stained dark brown (Fig. 5).

In patients with cervical cancer who did not experience recurrence, the following results were obtained: 11 (55%) of 20 VEGF-positive patients showed a weakly positive reaction. These findings indicate that reactions with low expression of the MPA reagent are associated with significantly reduced rates of tumor growth and metastasis. Conversely, reactions with high MPA reagent expression demonstrate increased probability of tumor growth and metastatic spread.

Analysis using the VEGF reagent revealed a strongly positive reaction in 11 (55%) of 20 patients. This suggests a substantially elevated risk of prolonged tumor growth and metastasis.



1. Strongly positive reaction. Squamous cell carcinoma G3. 2. Moderately positive reaction. Squamous cell carcinoma G1. 3. Weakly positive reaction of VEGF reagent in patients with cervical cancer without recurrence. IHC-Comp chromogen. No. 10. col40

Fig. 5. The degree of proliferative activity of the VEGF reagent in recurrent squamous cell carcinoma of the cervix.

Membrane protein CD34 – microscopic examination of CD34 membrane protein in the selected 20% of patients demonstrated the following pathological changes: squamous epithelial hyperplasia with atypical epithelial cells displaying pleomorphism, numerous atypical tumor cells, and multiple pathological mitoses. Vascular structures stained dark cinnamon-brown were observed in varying sizes, with 15-20 vascular profiles per field of view (Fig. 6).

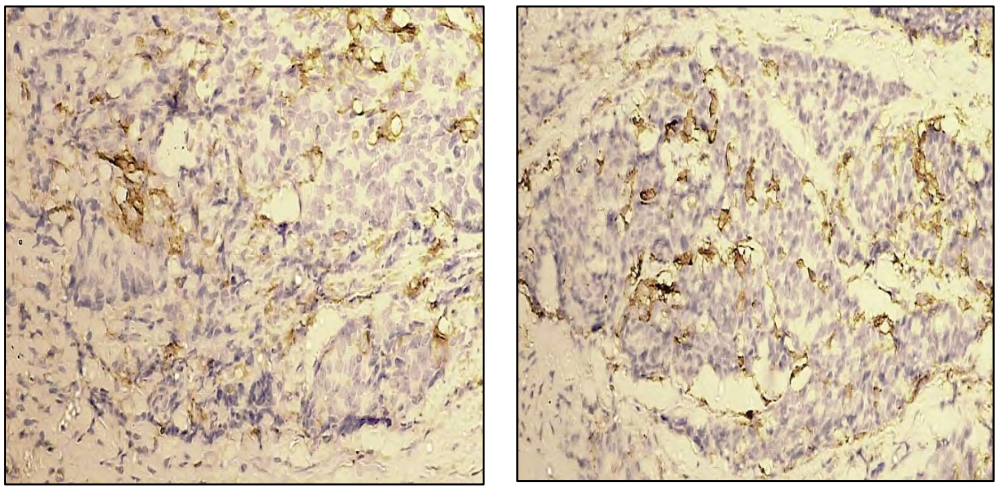
In cases of cervical cancer recurrence following complex or combined treatment, these microscopic changes were more pronounced (Fig. 7). Furthermore, the risk probability of distant metastasis in such cases was increased.

The microscopic examination of specimens from 20 selected patients revealed squamous epithelial hyperplasia, atypical epithelial cells with polymorphism in layers of varying shapes, and atypical tumor cells with prominent pathological mitoses. Dark brown blood vessels of differing sizes were observed, with a density of 15-20 vessels per field of view.

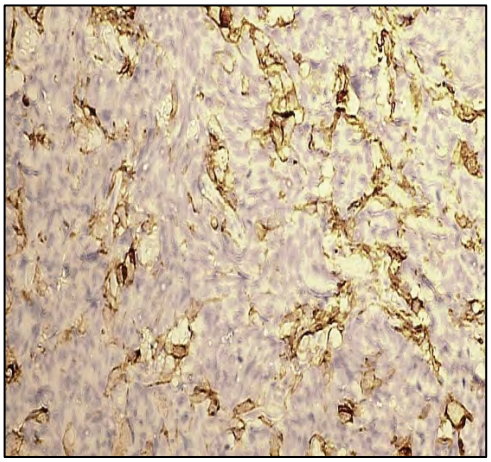
The subsequent investigation aimed to assess the outcomes of combined and complex treatment for squamous cell carcinoma of the cervix. The following data were obtained in cases with recurrence and long relapse-free periods:

In **G1 squamous cell carcinoma of the cervix** treated with combined therapy (radical surgery and combined radiation therapy), recurrence was predicted based on IHC results in 5 (25%) cases. These patients received two courses of PCT under the PP regimen (paclitaxel + carboplatin, with drug induction guided by topometric data) (Table 5). No disease recurrence or metastasis was detected in this group over 36 months.

In **G2 squamous cell carcinoma of the cervix** treated with combined therapy (radical surgery and combined radiation therapy), recurrence was predicted based on IHC results in 7 (35%) cases. Patients underwent three courses of PCT under the PP regimen (paclitaxel + carboplatin, with drug induction guided by topometric data). Two patients with extragenital pathology (postradiation thrombophlebitis of the lower extremities) were unable to receive chemotherapy, and recurrence was detected within 15+4 months.

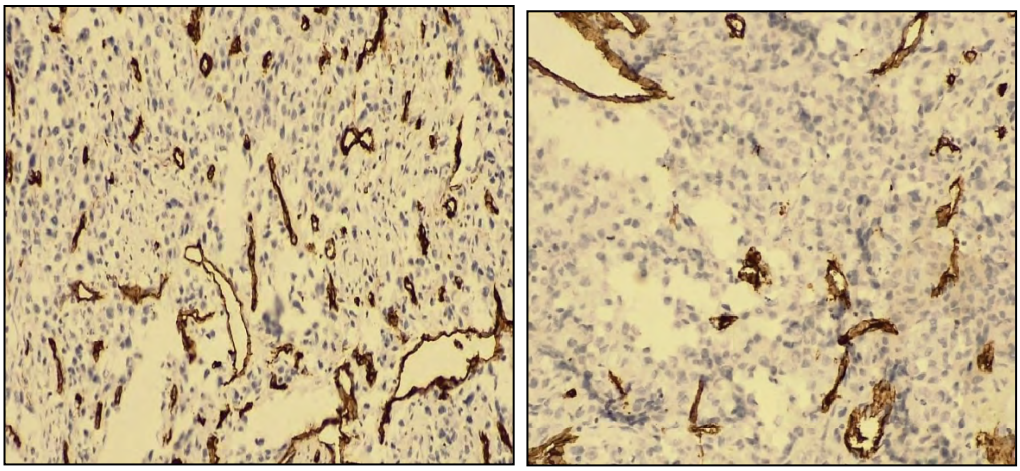


Squamous cell carcinoma G1 of the cervix Squamous cell carcinoma G2 cervical cancer moderate low positive reaction of CD34 reagent positive reaction of CD-34 reagent (vascular density 10-15). (vascular density 15-20). ICH – Comp chromagen. № 10. col40



Squamous cell carcinoma of the cervix. G-2. high positive reaction of CD-34 reagent (vascular density 30-40). ICH – Comp chromagen. № 10. col40

Fig. 6. CD34 results in patients with squamous cell carcinoma of the cervix.



1. A weakly positive reaction of CD34 reagent in patients with cervical cancer. (the density of vessels is 15-20). 2. A high positive CD34 response in case of recurrence of cervical cancer (30-40 vascular density). ICH – Comp chromagen. № 10. col40

Fig. 7. CD34 results in patients with squamous cell carcinoma of the cervix with disease recurrence.

Table 3

Immunohistochemical analysis according to the G-grade of the tumor.

	Ki 67	VGFR	CD34
G1	5 (25%)	2 (19%)	5 (25%)
G2	5 (25%)	7 (35%)	5 (25%)
G3	10 (50%)	11 (55%)	10(50%)

In **G3 squamous non-keratinizing cervical carcinoma**, recurrence occurred within 15+4 months in 10 (50%) cases after complex treatment, while in patients receiving combined therapy, this interval was 10+3 months.

According to the study data, lower degrees of differentiation in malignant cells correlate with decreased Ki67 expression, elevated VEGFR and CD34 levels, and a higher predicted risk of cervical cancer recurrence, observed in approximately 55% of the study cohort.

Conclusion

– The study findings demonstrate that higher proliferative activity (Ki67) in cervical cancer is associated with more favorable treatment outcomes and prognosis.

– Low VEGFR expression correlates with significantly reduced tumor growth and metastatic rates.

– Elevated CD34 levels are linked to an increased risk of cervical cancer recurrence and metastasis.

– The primary objective of immunohistochemical (IHC) research in clinical practice is personalized patient management to enhance treatment efficacy, achieve sustained remission, and prevent disease recurrence or metastasis.

– Comprehensive histopathological and immunohistochemical evaluation of tumor progression is a complex and time-consuming process; however, its diagnostic accuracy facilitates optimal therapeutic selection, treatment monitoring, and improved prognostic assessment.

– The results indicate that poorly differentiated malignant tumors exhibit more aggressive behavior, higher recurrence rates, and increased metastatic potential.

– Disease progression and treatment response vary significantly among patients within the same cohort, emphasizing the need for refined diagnostic approaches and individualized therapeutic strategies in cervical cancer management.

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ІМУНОГІСТОХІМІЧНІ ПРОГНОЗНІ ФАКТОРИ ПЕРЕБІГУ ТА РЕЗУЛЬТАТІВ ЛІКУВАННЯ РАКУ ШИЙКИ МАТКИ

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Резюме.

Рак шийки матки на сьогодні посідає третє місце за захворюваністю серед онкогінекологічних захворювань жіночої репродуктивної системи в Республіці Узбекистан. За даними літератури, плоскоклітинний рак становить до 77+3% випадків, аденокарцинома – 11%, саркома – 1%.

Мета дослідження: вивчення імуногістохімічних прогностичних факторів, що впливають на перебіг та результат лікування раку шийки матки.

Матеріали та методи дослідження. Ми проаналізували результати імуногістохімічного (ІГХ) дослідження 40 пацієнток з раком шийки матки, які лікувалися в Самаркандському відділенні Республіканського спеціалізованого науково-практичного медичного центру онкології та радіології протягом 2020-2023 років. До когорти увійшли 20 пацієнток з рецидивом захворювання після комплексного лікування та 20 пацієнток без рецидиву. Усі досліджувані випадки були гістологічно підтверджені як плоскоклітинний рак шийки матки (T1b-3aNxM0).

Результати. Імуногістохімічна оцінка маркерів Ki67, VEGFR та CD34 виявилася найбільш цінною для планування лікування та прогнозування захворювання. Дослідження демонструє характерні зміни в паттернах експресії Ki67, VEGFR та CD34 відповідно до ступеня злоякісності пухлини (G-класифікація).

Висновки. Комплексний гістопатологічний та імуногістохімічний аналіз раку шийки матки є технічно складною та трудомісткою процедурою. Однак його діагностична точність дозволяє оптимально підібрати лікування, контролювати реакцію на терапію та покращити прогноз захворювання.

Ключові слова: рак шийки матки, імуногістохімічне дослідження; ступінь проліферативної активності, рецидив; хіміотерапія.

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