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STUDY OF IMMUNOHISTOCHEMICAL MORPHOLOGIC FEATURES OF KIDNEY PERITUMOROSIS SITES IN KIDNEY ONCOLOGY (on the example of the Aral Sea region)

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Abstract: The article studies the features of immunohistochemical method in patients diagnosed with renal cancer and analyzes the results obtained.

Keywords: Renal cancer; morphology; immunohistochemical method; areas of renal peritumorosis.

Relevance. Experts from the World Health Organization and the International Agency for Research on Cancer (IARC) estimate that more than 500,000 new cases of kidney cancer are diagnosed each year worldwide. In 2020, kidney cancer ranked as the 16th most commonly diagnosed cancer worldwide, accounting for about 2.2% of all cancer diagnoses. In addition, kidney cancer is responsible for 1.8% of all tumor-related deaths, making it one of the most dangerous urologic tumors because of its 12% 5-year survival rate. Kidney cancer is considered one of the urgent problems facing modern medicine, the solution to which must be found, and also has a high risk due to an increase in incidence of 6-10% per year and high mortality of patients after treatment. The treatment of this disease is based on surgical intervention. In kidney cancer surgery, morphologic diagnosis is practiced, which is crucial for objectively determining the stage of the disease and determining further treatment tactics. The heterogeneity of tumor cell properties is the main source for the study of morphometric parameters of nucleus cells obtained from surgical material of renal cancer patients. Identification of phenotypic features of cells allows to develop a more objective grading of the tumor and objectify the prognosis of the disease. The heterogeneity of tumor cell properties is the basis for studying morphometric parameters of cell nuclei obtained from surgical material of kidney cancer patients. Identification of phenotypic features of cells allows to develop a more objective grading of the tumor and objectify the prognosis of the disease. On the other hand, knowledge of the pathogenesis of the disease ensures its timely treatment, increases medical, social and economic efficiency.

Purpose of the study. We aimed to study morphologic changes of renal peritumorosis sites in patients with renal cancer by immunohistochemical method in the population living in the Aral Sea area.

For this purpose, 63 patients who underwent surgery in 2017-2022 at the branches of RSPCC and R in Khorezm and Nukus were selected, micropreparations of 20 patients who underwent surgery with kidney cancer were re-examined for immunohistochemical examination, and areas of peritumorosis were re-examined immunohistochemically

Materials and Methods. Complex clinical and morphological data of 126 patients with kidney cancer treated in 2021-2023 in Khorezm branch of the Scientific and Practical Center of Oncology of the Republic of Uzbekistan were taken for the study. The mean age of the patients was 57.8±0.6 years (min 27; Max 77;) (Table 1). Mean tumor size-4.8±0.1 cm. (min - 1.2 sm; max - 14 sm; moda - 6 sm). The number of tumor

biomaterials taken for the study was 126. Material for morphological (histological) studies was taken and examined from the central and peritumor parts of tumors, as well as unaltered renal tissue.

Table 1. Distribution of renal cancer patients by age groups.

Indicators	18-44	45-59	60-74	75-90	total
%	22,2±3,93%	25,0±3,85%	41,6±4,39%	11,1±2,79%	100%
Absolute	n =28	n = 31	n=53	n = 14	n = 126

The study was investigated in groups according to clinical stages of tumors:

I clinical stage (T1N0M0) - 77 patients (61,1±4,34%);

II clinical stage (T1N1M0) - 35 patients (27,7±3,98%);

III clinical stage (T1N1M1, T2N1M1, T3N1M1) - 14 patients (11,1±2,79%).

Morphological evaluation of renal tumors was performed using the 2016 World Health Organization international classification.

Immunohistochemical, morphometric and statistical methods were used in the study.

Results of the study. Immunohistochemical study of pathomorphological malignant tumors is one of the innovative methods studied for the first time in our republic. The results of morphological study additionally, were carried out by immunohistochemical study of Ki67, which is currently recognized as the gold standard all over the world. P53, CD34, CD3 and CD20 angiogenic oracles were studied.

Immunohistochemical studies are an important step in modern morphological diagnosis and are based on the reaction between tumor antigens and antibodies visible by chromogen staining. These studies require several preparatory steps before the direct analysis, and their quality significantly affects the results of the analysis. Therefore, this verification requires sequential implementation in several steps (Table 2)

Table 2. Stages of immunohistochemical (IGX) examination.

№	**Stages of immunohistochemical study**	**Reagents**	**time**
1	Preparation of 4 µm thick slices	Polylized glass	
2	Slice drying		24 hours at room temperature
3	Thermostat drying		At t0-55-60C - 60 min
4	Dewaxing	Orto-Ksilol	10 minutes 3 times
5	Dehydrothesizing	Spirt 96%	3 minutes 3 times
6	Rehydration	Distilled water	10 min
7	demask	Demasking Bufer	30-40 min in water t^0-98C
8	Washing	solution tris - bufer (pH=7,5).	5 min
9	Blocking endogenous peroxidase	Vodorod perekis 3%	5 min
10	demask	Distilled water	3 min
11	Appearance and incubation of primary (basic) antibodies	Specific antibodies	20-30min
12	demask	solution tris-bufer (pH=7,5).	5 min
13	Appearance and incubation of primary (basic) antibodies	Vizual tzm	20-30min
14	demask	Раствор tris-bufer (pH=7,5).	5 min
15	Determination by diaminbenzidine	DAB-xromogen	5 min
16	demask	Дистиллированная вода	3 min

17	Coloring	Gemotoksilin Mayera	5 min
18	demask	Running water	1 min
19	Dehydrothesizing	Spirt 96%	2 times 5 min.
20	Despiritualization	Orto-ksilol	2 times 5 min.
21	Conclusion	Balm, mirror coating	

When conducting studies by immunohistochemical method, proper use of reagents is considered important, and the following reagents were selected for this purpose (Table 3).

Table 3. Reagents used in immunohistochemical study

№	**Випы реагентов**	**Количества**
1	Reagent Ki67	20
2	Reagent P53	20
3	Reagent CD34	20
4	Reagent CD3	20
5	Reagent CD20	20

This examination is performed to analyze kidney cancer patients. In order to identify molecular structures in cells, to study the arrangement of cells, to study the dissemination or histogenesis of tumor diseases, to observe these processes in the development of precancerous processes, to identify prognostically observed disease aosrates, to determine tumor stages, to determine treatment tactics, to dynamically monitor and control treatment processes, and to identify risk groups from which tumor diseases may originate, this screening for uchuli is essential.

Areas of peritumorosis in renal cancer - 20 patients were selected for this examination. The results obtained in all patients were evaluated in percentages as an indication of the proliferative activity of Ki67 - tumor cells. The results were evaluated according to mild, moderate and severe positivity. Of the 20 patients, 12 (60±10.95%) had moderate positivity and 8 (40±10.95%) had mild positivity. In our study, a high positive response to this reagent was not observed. By microscopic appearance: kidney cells have polymorphosis, consist of hyperchromic nucleus and pathological mitoses, angiomatosis in peritumorosis zone, sclerosis consist of tumor cells with lymphocytic infiltration around blood vessels, tumor cells in peritumorosis zone are stained dark brown (Fig. 1).

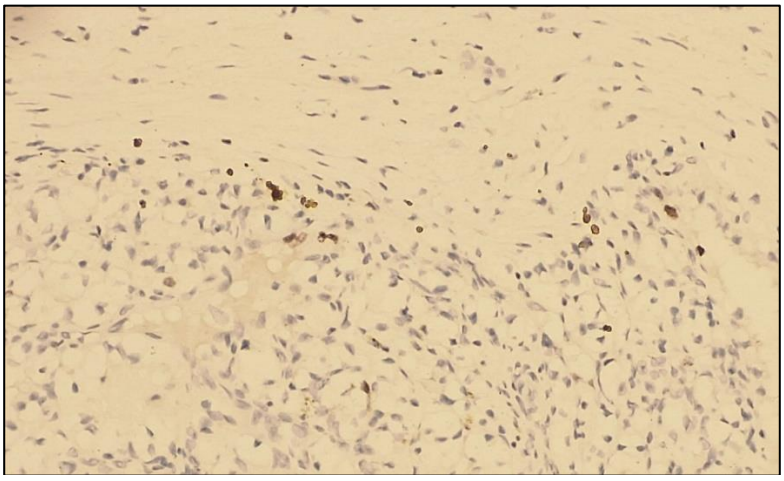

Figure 1. Mean degree of Ki67 reagent positivity in the peritumorosis zone of renal cancer. IGX – Dab xromagen. Ob10xok40.

Renal cancer in the peritumorosis zone - 20 patients were selected for the purpose of p53 reagent screening. The results obtained in all patients were evaluated through mild, moderate and severe positivity results. Negative reaction in 15 (75 ± 9.68%) of 20 patients, low positive reaction was observed in 5 (25 ± 9.68%) patients, no moderate or high positive reaction was observed during examination. On microscopic appearance: renal cells are polymorphized, consisting of hyperchromic nucleus and pathological mitoses, tumor cells undergo angiomatosis in the peritumorosal zone, tumor cells undergo polymorphism in ozmicdor, lymphocytic infiltration around vascular sclerosis, tumor cells in the peritumorosal zone have a low degree of dark brown color (Figure 2).

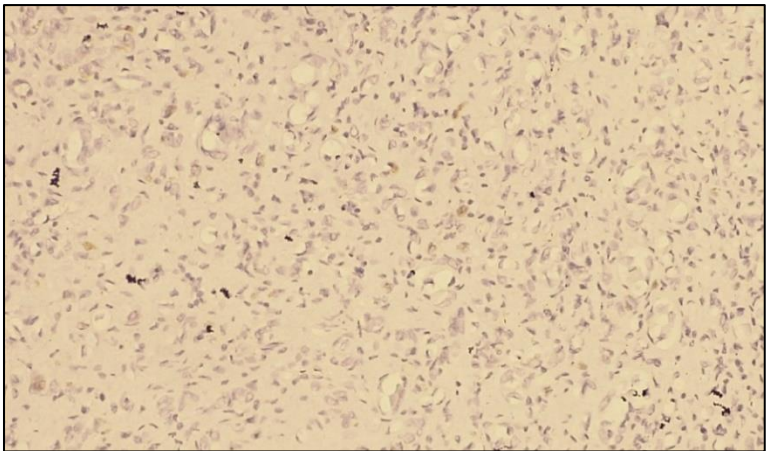

Figure 2. Mutant oxil r53 with low positivity in the peritumorosis zone of renal cancer. IGX – Dab xromagen. Ob10xok40.

20 patients were selected to evaluate blood vessel density using CD34 reagent in the peritumor area affected by renal cancer. The results obtained in all patients using the CD34 reagent were evaluated by examining the richness of the tumor conjunctiva and its relationship to the pattern of tumor metastasis. The results obtained were evaluated according to the negative and positive response results. In all 20 patients, a positive response was observed in 100% of patients. When viewed under a microscope in one field of view, the density of the tumor and 20-30 vessels of different volumes in its peritumorosal zone were determined. The high density of blood vessels in renal cancer indicates a higher degree of infiltration of these tumors into surrounding tissues and a higher degree of spread (MTS) to associated organs. No cases of adverse reaction were observed. (Figure 3).

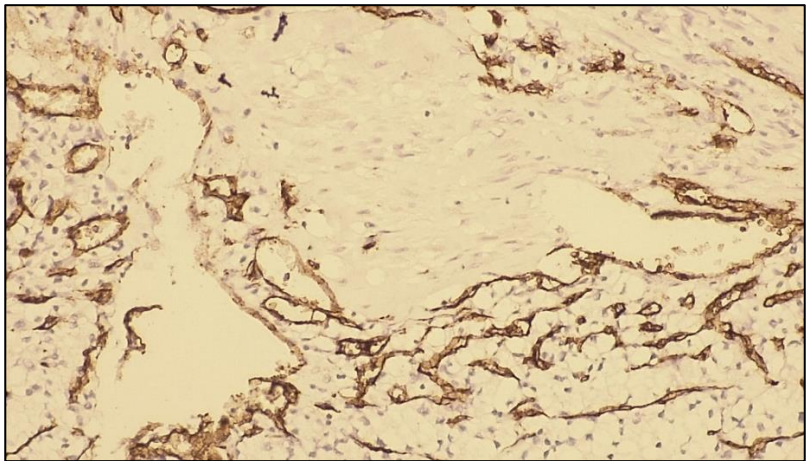

Figure 3. CD34 reagent positivity in the peritumorosis zone of renal cancer. IGX – Dab xromagen. Ob10. Ok40.

CD3-immunological indicators quantitative indicators of immunoregulatory cellular composition showed: significantly higher levels of T-lymphocyte inducers were observed in tumor tissue. Indications Along the resection line, proliferative activity of T lymphocytes was observed in large numbers around peritumorotic areas. Analysis of kidney tissue obtained during tumorigenesis for immune cell content showed a reliable location of T-lymphocytes (CD3+) relative to the resection line and peritumoral zone. It should be noted that a large number of natural killer cells are noted in the peritumoral zone. When viewed under a microscope, the tumor and its peritumorosis zone showed high infiltration of T-lymphocytes in the surrounding tissues and in the peritumorosis zone of tumors. (Figure 4).

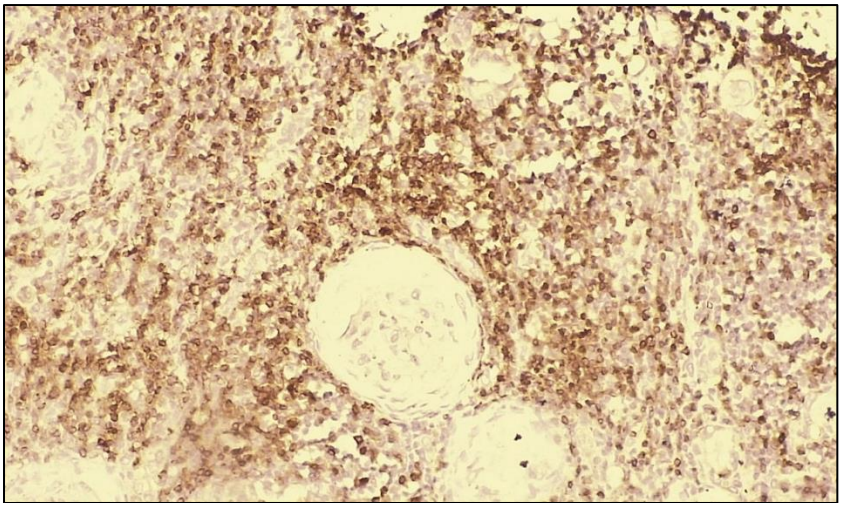

Figure 4. High CD3 reagent positivity in the area of peritumorosis in renal cancer. IGX – Dab xromagen. Ob10. Ok40.

The results obtained in all patients were evaluated for mild, moderate and severe positivity. High positivity was observed in 12 (60%) of 20 patients and moderate positivity in 8 (40%) patients, while no low or negative positivity was observed.

CD20 - (C - lymphoblast antigen) is a protein and correlator found on the surface of B-lymphocytes and is a product of the human ms4a1 gene. It is known from the literature that V is involved in lymphocyte activation and proliferation. CD20 V is specific for lymphocytes. It is also present on the surface of malignant cells in most B-cell lymphoproliferative diseases.

In renal cancer, high levels of V-lymphocyte proliferative activity around peritumorotic areas were observed along the resection line. Analysis of kidney tissue obtained during tumorigenesis for immune cell content showed a reliable location of V-lymphocytes (CD20) relative to the resection line and peritumoral area. It should be noted that a large number of natural killer cells was noted in the peritumoral zone. When viewed under a microscopedla mainly proliferative activity of V-lymphocytes in the peritumoral zone was observed (Fig. 5).

The results obtained in all patients were as follows: high positive response was observed in 50% (N=10) of 20 patients and medium positive response was observed in 25% (N=5), while a process of low positive response was observed in 25% (N=5) of patients.

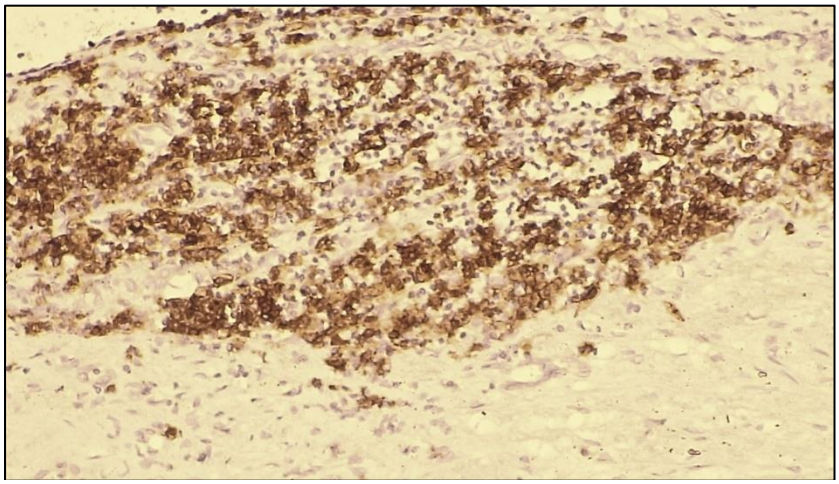

Figure 5. High CD20 reagent positivity in the area of peritumorosis in renal cancer. IGX – Dab xromagen. Ob10. Ok40.

As seen above, cells of the immune system play an important role in the development of kidney cancer. Thus, there is an association between survival of renal cancer patients and lymphocyte infiltration with changes in oncomarker expression levels. Significant infiltration by lymphocytes and histiocytes along the invasive margin of tumor tissue is probably one of the important factors characterizing the duration of survival, since altered activity of immunocompetent cells, cell communication, plays an important role in providing immunological protection during malignant growth. CD3 T-lymphocytes CD20 B-lymphocytes direct infiltration in the stroma and microenvironment of tumor tissue determines the best prognosis, as it provides high activity of the immune system and immune cells, which indirectly exercise systemic immunological control over the tumor process. The presence of pronounced lymphocytic infiltration probably explains a more favorable outcome of the disease, since increased activity of cellular immunity is one of the alternative ways of stimulation of the immune system providing antitumor immunity.

Conclusions.

Thus, based on the use of modern immunohistochemical and pathomorphologic methods of investigation in renal cancer, important criteria for its feasibility in renal cancer patients were determined.

The results of the study of quantitative indices of the immune system in patients with renal cancer showed that in tumor tissue T - lymphocytes are largely auxiliary inducers, which, in turn, is an immunoregulatory state. In this case, patients with a resection line of at least 1-2 cm and relative to the peritumoral zone had a high index of the number of T-lymphocytes (CD3+) and B-lymphocytes (CD20+).

Evaluation of morphologic, immunohistochemical and immunologic criteria of renal cancer showed their interrelation, since changes in the activity of immunocompetent cells, cell communication, play an important role in providing immunologic protection in malignant growth.

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