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Original Article

Evaluation of New Immunohistochemical Approaches for the Study of Kidney Tumors in Geriatric

Pavlova, T. V[1 *], Pilkevich, N. B[1], Bessmertnyi, D. V[1], Pavlov, I. A[2], Atiakshin, D. V[3], Pavlova, L. A[1]

1. Department of Pathology, Belgorod State University, Belgorod, Russian Federation
2. Urological Department, Belgorod Cancer Clinic, Belgorod, Russian Federation
3. Voronezh State Medical Academy Named after N. N. Burdenko, 394036, Voronezh, Russian Federation

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Corresponding Author: pavlova@bsu.edu.ru

Abstract

Kidney malignancies are among the most deadly genitourinary tumors. It is more common in males and is often seen in people aged 60-70 years old. The incidence rate of kidney cancer seems to be increasing. One reason for this may be the fact that imaging techniques, such as computed tomography scans are more commonly used. These tests may lead to the accidental detection of more kidney cancers. Fortunately, kidney cancer is often detected in the early stages, when the tumor is small and confined to the kidney. The objective of this study was the development of new diagnostic immunohistochemical methods. Clinical examination material of 134 people, including 94 (70%) males and 40 (30%) females, were used in this study. Immunohistochemical staining of tryptase was carried out in compliance with the requirements using Anti-Mast Cell Tryptase antibodies. Goat anti-mouse antibodies #AS-M1-HRP were used as secondary antibodies, visualized with ImmPACTTM DAB Peroxidase Substrate Kit (#SK-4105) according to the instructions of the manufacturer. The nuclei were counterstained with Mayer's hematoxylin, and the sections were embedded in a permanent mounting medium. The immunohistochemical study showed an increase in both tryptase- and chymase-positive mast cells in the renal parenchyma, compared with the control group. The number of mast cells with tryptase expression directly in the tumor was significantly less than the peritumoral localization. A similar pattern was observed for chymase-positive mast cells as the content of the tumor was more than 10 times higher than the intratumoral arrangement. The histological and immunological characteristics did not differ in different age groups. The immunohistochemical method of research in the diagnosis of renal tumors plays an important diagnostic and prognostic value. It can assist pathologists in difficult and ambiguous cases to correctly diagnose renal tumors. This will make it possible to prescribe the correct treatment and predict the course of malignant tumor growth in patients.

Keywords: Kidneys, Metastases, Oncourology, Trace elements

1. Introduction

Kidney tumor is among the 10 most dangerous diseases, which is more common withinthe age range of 50-70 years old and is one of the most common cancers (1, 2). It accounts for 2-4% of malignant cancers. In urology, it is one of the three most important diseases. Moreover, it should be mentioned that the prevalence of kidney tumors is significantly higher in males. Smoking plays a significantrole in the incidence ofkidney tumors. The first sign is usually the presence of blood in the urine, at which time the doctor finds thetumor by performing an ultrasound (3, 4). There are cancer cells in all humans that die spontaneously or lose their destructive effect under the

influence of the immune system (5-7). A programmed celldeath (apoptosis) plays an important role in the spontaneous death of cancer cells (8, 9).

In recent years, both in Russia and globally, there has been an increase in the incidence rate of malignant tumors, including kidneys, which attracts the attention of various specialists (i.e., urologists, oncologists, and pathomorphologists). Malignant kidney tumors rank 10th in the cancer registry among all neoplasms. According to the literature, it occurs in patients aged 30 years and older (10, 11). Moreover, it should be noted that men suffer almosttwotimes more often than women (10-12).

In recent years, renal tumors (especially malignant ones) have been more often diagnosed with delay**,** when the pathological process already had signs of aggressive growth: tumor growth into the vessels, which further leads to the formation of metastases and shortens the survival time of patients(13, 14). The cause of most kidney cancers is unknown. According to medical findings, the genetic material/DNA in some kidney cells is mutated, which causes kidney cancer. Changes in this substance cause cells to grow and divide rapidly. The accumulation of these abnormal cells causes a tumor that can involve nearby organs in addition to the kidney, which is called metastasizing the involvement of organs farther away from the kidney (15-17).

Kidney cancer has different types, such as renal cell carcinoma, urothelial carcinoma, sarcoma, Wilms tumor, and lymphoma (18, 19). The prognosis of malignant renal tumors, as well as tumors of other localizations, depends on the presence or absence of regional and distant metastases on the stage and histological variant of the disease, which cannot be differentiated without morphological examination (12-14).

About 1% of all oral cancers are metastases from primary tumors that occur in other parts of the body. The most common kidney tumor is considered a malignant epithelial tumor -renal cell carcinoma (RCC) - 80-90% of all kidney tumors. The RCC has a large number of histological variants of the structure, the most common of which is the light-cell variant. It must be mentioned thatthe papillary, chromophobic, and other variants are less common (20-22). The immunohistochemical study is currently considered one of the most informative studies as it allows the most accurate determination of both the histogenesis of the tumor and the establishment of the degree of its differentiation (20, 22).

At the present stage of development of morphology, there are no difficulties in obtaining antibodies to almost any antigen. By studying specific molecules, immunohistochemistry provides information about the functional state of the cell andits interaction with the microenvironment to establish the phenotype and particular tissueto which the cell belongs.

The epithelial nature of the kidney tumor can be confirmed by antibodies, such as the epidermal growth factor receptor and alpha-Methylacyl-CoA racemase (AMACR). The AMACR is a specific marker for detecting prostate cancer but it also responds to malignant epithelial tumors of other localizations (14, 23, 24). Antibodies of proliferation and apoptosis help establish tumor differentiation P53, P63, Ki-67, and proliferating cell nuclear antigen: the more intense the expression of proliferation markers is, the more aggressively the tumor behaves and, accordingly, the lower differentiation it has. Therefore, each kidney tumor, as well as each variant of RCC, has its own original combination and intensity of staining with antibodies. However, there is still no single scheme forthe differentiation of various variants of kidney tumor growth.

In this regard, this study aimed to develop new diagnostic immunohistochemical methods.

2. Material and Methods

This research was based on the material obtained in 2015-2020 at Belgorod Oncological Dispensary, St. Joseph Belgorod Regional Clinical Hospital, and Belgorod Pathologicanatomical Bureau Belgorod, Russia. The study of the material, analysis, and

processing of the obtained results were carried out at the Department of Pathology at the Scientific, Educational, and Innovation Center of Nanostructured Materials and Nanotechnologies of Belgorod State University, Belgorod, Russian Federation.

The study involved the examination of 134 people, 94 (70%) of which were male. Groups were formed according to age and nosological criteria (Table 1).

Table 1. Patients with kidney pathology.

Control (n=20)		Middle age (40-49 years) Elderly age (60-83 years)		n=10 n=10
Pathology of the kidneys, bladder (n=114)	Kidney cysts (n=22)	Middle age (41-55 years) Elderly age (60-78 years)		n=10 n=12
	Kidney cancer (40 females and 52 males) n=92	Middle age (40-55 years) (n=43)	Stage I (T_1 N_0 M_0) Stage II (T_1-T_2 N_0M_0) Stage III (T_1-T_3 N_1-N_2) Stage IV (T_1-T_3 N_1- N_2 M_1)	n=10 n=12 n=13 n=8
		Elderly age (61-79 years) (n=49)	Stage I (T_1 N_0 M_0) Stage II (T_1-T_2 N_0M_0) Stage III (T_1-T_3 N_1-N_2) Stage IV (T_1-T_3 N_1- N_2 M_1)	n=10 n=18 n=16 n=5

All the subjects had neither exacerbation of chronic diseases nor severe concomitant somatic pathology. Moreover, the patients of the control groups did not present urological complaints and purposefully did not turn to specialists of this profile.

For histological examination under light microscopy, specimens were collected from various parts of the kidneys, fixed, embedded in paraffin, and sectioned on a microtome, followed by staining with hematoxylin and eosin. Finally, they were examined and photographed in a Topic-T light Ceti microscope. This study analyzed the expression of tryptase and chymase in mast cells associated with kidney cancer. The kidney cancer and control groups consisted of five and six patients, respectively.

Immunohistochemical staining of tryptase in compliance with the necessary requirements (14) was carried out using Anti-Mast Cell Tryptase antibodies (clone AA1, #ab2378, dilution 1:3000), chymase – Anti-Mast Cell Chymase antibodies (clone CC1, #ab2377, 1:2000). Goat anti-mouse antibodies #AS-M1-HRP were used as secondary antibodies, visualized with ImmPACTTM DAB Peroxidase Substrat Kit (#SK-4105) according to the instructions of the manufacturer. The nuclei were counterstained with Mayer's hematoxylin, and the sections were embedded in a permanent mounting medium.

The expression profile of kidney mast cell proteases was assessed usingdouble immunolabeling (14). Simultaneous staining was performed with AntiMast Cell Tryptase antibody EPR9522 (ab151757, dilution 1:1000) and Anti-Mast Cell Chymase antibody (clone CC1, #ab2377, 1:1000) in accordance with the standard protocol. The primary antibodieswere detected using Goat Anti-Mouse IgG H&L (ab97035) and Goat Anti-Rabbit IgG H&L (ab150077) conjugated with Cy3 and Alexa Fluor 488 fluorochromes, respectively. Next, the nuclei were counterstained with DAPI (5 μg/ml phosphate-buffered saline; Sigma) for 15 sec, washed with phosphate buffer, and the sections were embedded in an anti-fluorescent mounting medium.

Sections were examined using a ZEISS Axio Imager. A2 microscope with an image recording system, including a Camera Axiocam 506 color digital

camera and a Camera Axiocam 503 mono monochrome camera. The images obtained were processed using ZEN software (version 2.3) (Carl Zeiss, Germany). The volume of the kidney mast cell population was determined in conventional visual fields using a ×40 lens, which was at least 50 cells to obtain a representative data array. After the performed planimetric analysis, to facilitate the perception of the obtained digital array, the results were adapted to a tissue area of 1 mm^2.

To identify somatic pathology, diagnostic measures were carried out, followed bya collection of complaints and anamnesis with a targeted survey of systems and organs, physical examination, as well as laboratory and instrumental research methods. These methods includedgeneral blood test, general biochemical blood test, general urine analysis, electrocardiogram registration, study respiratory function, and chest x-ray.

In the presence of kidney pathology in patients, to establish and clarify the diagnosis, laboratory and instrumental examination werecarried out, including a comprehensive ultrasound examination of internal organs, lymph nodes, microbiological examination of urine, scintigraphy of the bones of the skeleton, and if necessary, computed tomography. This study included patients with histological verification of the disease.

3. Results and Discussion

Histological examination of the obtained kidney biopsies showed that the majority (93%) of the tumor was represented by clear cell carcinoma. It was a malignant tumor with a thin vasculature, consisting of cells with light or eosinophilic cytoplasm. It was predominantly solitary and located in the renal cortex (23, 24). Macroscopically, for the most part, it was yellow, in the form of a node with clear boundaries and a pseudocapsule. Often it was characterized by secondary changes in the form of foci of necrosis, hemorrhages, and calcifications (19).

Invasion of the tumor into the perineal tissue and/or its ingrowth into the renal vein was observed in about 41% of cases which is similar to the results of previously reported research (19-22). The rest of the cases (7%) were papillary cancer. In half of the cases, a multifocal variant was observed,and it should be noted that the multifocal variant was more common in men. Moreover, the meanage of patients was 53-65 years, and it should be mentioned thatthefindings were in linewith those of previously published works (18-20). Histologically, for the most part, it contained papillary structures of small cells with scanty cytoplasm and small nuclei. In two cases, larger cells with well-defined eosinophilic cytoplasm, large nuclei, and prominent nucleoli were observed. It should be mentioned that the histological picture did not differ in different age groups.

This immunohistochemical study showed an increase in both tryptase- and chymase-positive mast cells in the renal parenchyma, compared with the control group, which is in agreement with previously published works (21-24). At the same time, the activity of degranulation of proteases by mast cells increased, which was morphologically manifested by the expansion of protease-positive pericellular staining of structures of the tumor microenvironment. Simultaneously, the number of mast cells with tryptase expression directly in the tumor was significantly less than in the peritumorallocalization (20-24). A similar pattern was observed for chymase-positive mast cells, the content of which around the tumor was more than 10 times higher than in the intratumoral arrangement.

A detected protease profile indicateda significant increase in the expression of chymase in the mast cell population, with the highest intensity in the peritumoralregion.This increasewas reflected in an increase in the number of mast cells with simultaneous expression of proteases. The obtained data indicated that the development of kidney cancer, in general, is accompanied by an increase in both tryptase- and chymase-positive mast cells in the renal parenchyma, compared tothe indicators of the control comparison group (Figure 1). It is noteworthy that the immunological characteristics in different age groups did not differ.

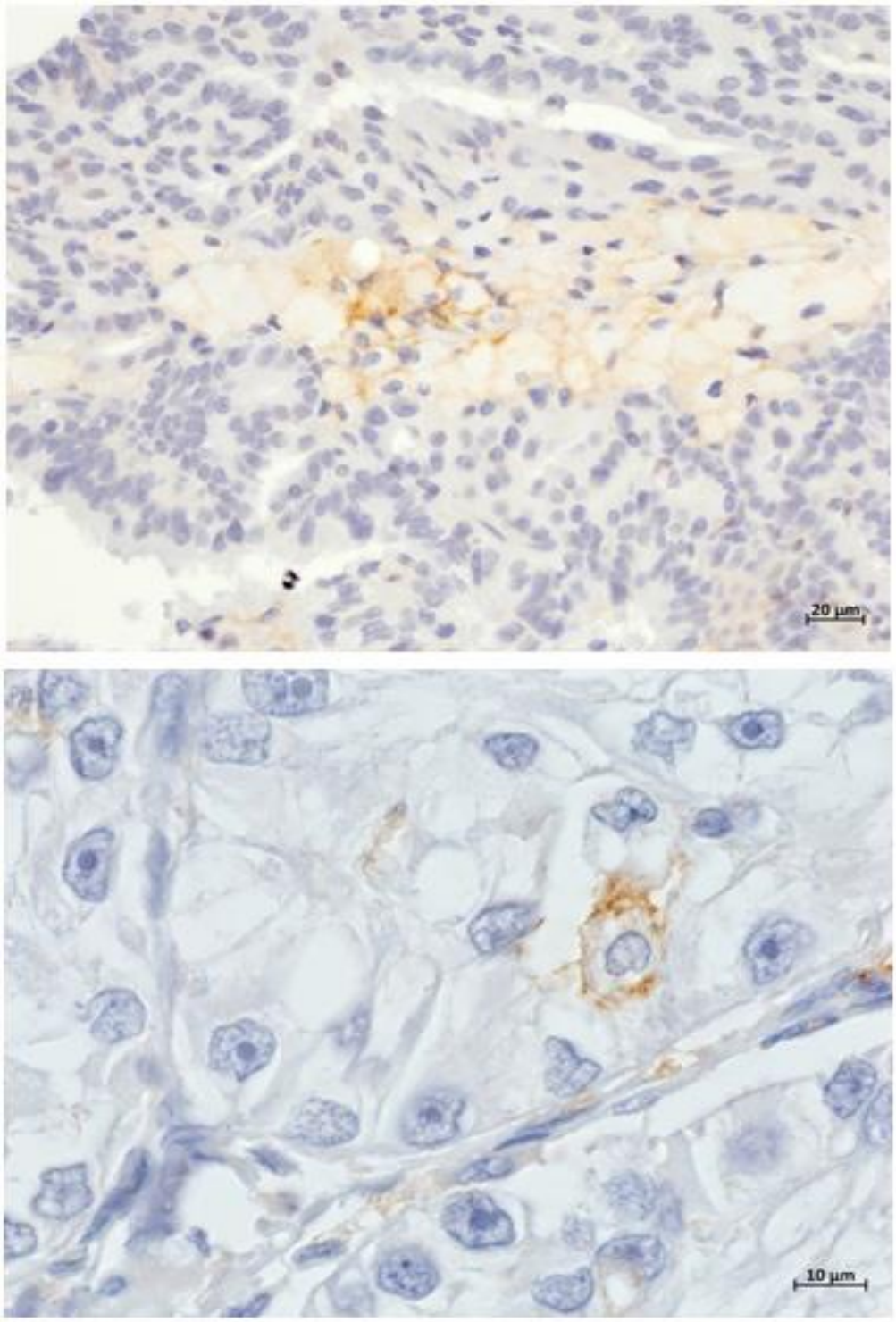

Figure 1. Tryptase-positive mast cells in kidney cancer.
Fixation with 19% neutral formalin. Immunohistochemical staining for tryptase.

Therefore, the immunohistochemical method of research in the diagnosis of renal tumors hasan important diagnostic and prognostic value. It can assist pathologists in difficult and ambiguous cases to correctly diagnose renal tumors. This makes it possible to prescribe the correct treatment and predict the course of malignant tumor growth in patients.

The development of kidney cancer is accompanied by an increase in the population of tryptase- and chymase-positive mast cells in the organ. Evaluation of the expression level of tryptase and chymase reflects the invasive ability of tumor tissue and can be a pathomorphological criterion for the aggressiveness of kidney cancer. Determination of the prognostic role of the number of mast cells in the kidney requires considering their histotopographic localization in the organ. The histological and immunological characteristics did not differ in different age groups.

Authors' Contribution

Study concept and design: T. V. P.
Acquisition of data: N. B. P.
Analysis and interpretation of data: D. V. B.
Drafting of the manuscript: I. A. P.
Critical revision of the manuscript for important intellectual content: D. V. A.
Statistical analysis: L. A. P.
Administrative, technical, and material support: T. V. P.

Ethics

The present study was approved by the Ethics Committee of Belgorod State University, Belgorod, Russian Federation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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