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Research Article

Innovative methods of atomic force microscopy in oncology diagnostics

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ABSTRACT

The purpose of this work is to study the morphological forms of erythrocytes, and also pathomorphological changes in prostate gland and kidneys with the use of atomic force microscopy (AFM) during oncological processes. **Methods**: Morphological properties of erythrocytes were studied by the means of hemoscanning and erythrocyte measuring. AFM was produced by us in the probe laboratory Ntegra-Aura (Russia). The studies were carried out in contact modes of intermittent or constant profile using commercial Si or SiN cantilevers (NSG01, NT-MDT, Russian Federation) under the conditions of low atmospheric vacuum. The processing and the implementation of AFM images were formulated using the software "NOVA" (NT-MDT, Russian Federation) and "ImageAnalysis" (NT-MDT, Russian Federation). **Results**: The decrease of discocytes was observed, and the number of transitional, pre-hemolytic and degenerative forms of erythrocytes increased. During the study of the external level of erythrocytes using AFM, it was established that the depth of the discocyte cavity counted by the study of a cell profile changed significantly. On the average, it made 0.48 ± 0.15 μm, which was significantly different from the control group. During the study of prostate cancer, it was found that with the progression of the disease, the contacts between cells of 0.5 μm, which can reach up to 3-4 μm, exceeding the cell sizes are more common. Besides, the number of tumor cell clones on the vessel endothelium increased. Conclusions: the result of the study showed that in the course of oncological processes the change of erythrocyte cytoarchitectonics was observed. The modified cells are partially functionally inferior and can not fully fulfill their most important oxygen transport function. Pathomorphological changes in a prostate gland revealed the deformed glands with branched structures, from which the papillae formed by atypical polymorphic cells with weak connections between them.

KEY WORDS: atomic force microscopy, oncodiagnosis, prostate cancer, kidney cancer, erythrocytes

[I] INTRODUCTION:

The problem of cancer remains extremely relevant, despite all the achievements of fundamental clinical medicine. The increase in morbidity, certain difficulties in timely diagnosis and the choice of tactics for adequate medical interventions encourage the identification of malignant tumors occurring in urological practice in a special category, combining them with the term "oncourology". The subject of oncourology consists of kidney, urinary tract and male genital organ neoplasms with a variety of anatomical localization and histological forms of detected tumors, the peculiarity of clinical symptoms and the presentation of these tumors in various types of diagnostic studies [1].

The most common nosological forms in oncourological practice are prostate cancer, bladder cancer, kidney parenchyma cancer, the cancers of renal and urinary system, urethral cancer, and testicular tumors are less common [2].

In most industrialized countries, prostate cancer becomes the most frequent malignant tumor among men - the incidence of prostate cancer in Eastern and Northern Europe makes 65 cases per 100 thousand, and the death rate is 26 cases per 100 thousand people a year. The rates of

prostate cancer incidence, 3% per year on the average are particularly disturbing ones. The latter circumstance makes it possible to predict the increase of annually registered cases in 2 times by 2030 [1, 3]. The cancer of the kidney parenchyma constitutes about 2% of the total number of malignant neoplasms and occupies the third place after prostate and bladder neoplasms and also shows the tendency of disease incidence increase [4-6].

Currently, 190,000 new cases of kidney cancer are detected annually in the world on the average, and, almost 1/3 of patients die because of tumor. The number of detected urinary tract tumors in the world reaches 260,000 new cases per year, so that the problem of diagnosis and the treatment of these patients continues to occupy a significant place in oncourology [1].

Due to the wide prevalence and social importance the range of research and diagnosis in oncology increases, including morphological ones [7, 8].

Atomic force microscopy (AFM) method can be used effectively in the study of surface microrelief. With its help it is possible to observe all possible features of a structure at pathological conditions [9-11]. AFM Atomic force microscopy (AFM) provides the obtaining of surface ultrastructure 3D images with molecular resolution in real time. In recent years the status of one of the main methods of research has been fixed for this method, which makes it possible to perform the first steps in human nanopathology [12, 13]. In this regard, the purpose of our study was the study of the morphological forms of erythrocytes using atomic force microscopy (AFM), as well as pathomorphological changes in a prostate gland and kidneys during cancer processes.

[II] MATERIALS AND METHODS

In the framework of the performed work, 198 men and women of different ages were examined, divided according to the nosological criteria into 2 groups. The 1st group included 110 men with diagnosed prostate cancer (PC) and benign prostatic hyperplasia (BPH). The second group included 88 men and women suffering from kidney pathology: kidney cancer (KC) and kidney cysts. The control group consisted of 70 almost healthy men and women.

The object of the study was represented by various tissues of oncological patients: blood samples from the vein and the tissues from postoperative biopsy.

2.1. The study of erythrocyte morphological characteristics

Native red blood cells were the material for the study. Venous blood was taken in the morning at an empty stomach in vacuum test tubes. During the first 30 minutes, the procedure for consistent washing of the erythrocytic mass and the formation of blood smears were carried out. The morphological properties of erythrocytes in the obtained samples were studied by the means of hemoscanning and erythrocytometry. During the description of the morphological characteristics, the erythrocyte population was divided into the main groups: 1) discocytes; 2) transitional forms (capable of reverse transformation): ellipses, discocytes with crest, flat discs, the discocytes with outgrowth, the discocytes with multiple outgrowths, the erythrocytes in the form of "mulberry berry"; 3) pre-hemolytic forms (with the changes bearing irreversible character): domed, spherical, in the form of a "blown out ball"; 4) degenerative forms.

2.2. The study of pathomorphological changes in cells and tissues

Cells and tissues were analyzed using atomic force microscopy (AFM). The samples were viewed and photographed after a brief fixation in formalin solution. All this makes possible the use atomic force microscopy (AFM) as an express method. Besides, paraffin blocks can be used to study the samples, especially if there is a need to study the slices obtained from them in a light microscope to select the required areas. Atomic-force microscopy (AFM) was produced by us in the probe laboratory Ntegra-Aura (Russia). The studies were performed in the contact modes of intermittent or permanent profiles using commercial Si or SiN cantilevers (NSG01, NT-MDT, Russian Federation) under the conditions of low atmospheric vacuum. The processing and the implementation of AFM images were formulated using "NOVA" software

(NT-MDT, Russian Federation) and "ImageAnalysis" (NT-MDT, Russian Federation).

[III] RESULTS AND DISCUSSION:

3.1. The study of red blood cell morphological characteristics: The results of erythrocyte morphological characteristics study showed that the percentage ratio of the main groups in the control group is the following: discocytes - 86.67 ± 0.80%, transitional forms - 10.00 ± 0.52%; Prehemolitic - 3.00 ± 0.37% and degenerative forms of erythrocytes - 0.33 ± 0.07% (Table 1).

Table 1. Percentage ratio of morphological forms of erythrocytes in the pathology of the prostate gland

Morphological Forms of erythrocytes	Control group	Patients with benign prostatic hyperplasia	Patients with prostate cancer
Discolets	86.67±0.80	81.67±1.20*	71.67±2.53**
Reversibly changed (transitional)	10.00±0.52	15.00±1.15*	23.17±1.89**
Irreversibly changed (Prehemolithic)	3.00±0.37	2.50±0.56*	4.00±0.77**
Degenerative forms	0.33±0.07	0.83±0.05*	1.16±0.05**

Note: * $p<0.05$ in comparison with the control group

** $p<0.01$ reliability of differences in comparison with benign prostatic hyperplasia (BPH)

The increase of reversibly altered erythrocyte number was observed predominantly due to discocytes with multiple outgrowths. Also this group had single dome-shaped erythrocytes and the erythrocytes in the form of a "mulberry" and a "deflated ball".

The study of the morphological state of erythrocytes in the first group of patients with the diagnosed prostate cancer (PC) and benign prostatic hyperplasia (BPH) as compared with the control group showed that the patients with benign prostatic hyperplasia (BPH) had a reliable decrease ($p<0.05$) of discocyte number by 5.77% and the number irreversibly altered red blood cells by 16.67%. At the same time, there was a significant increase of reversibly altered erythrocytes in 1.5 times, and the increase of erythrocytes with degenerative forms in 2.5 times.

A detailed analysis of erythrocyte architectonics in the group of patients with PC reflected the morphological heterogeneity of the erythrocyte population. A significant decrease of discocytes was observed by 17.31% (71.67 ± 2.53%) in comparison with the control group and by 12.25% as compared with the patients who had benign prostatic hyperplasia (BPH).

The results of morphological forms of erythrocytes study among the patients with kidney pathology are presented in Table 2.

Table 2. Percentage ratio of morphological forms of erythrocytes among the patients with renal pathology

Morphological forms of red blood cells	Patients with kidney cysts	Patients with Kidney cancer
Morphological Forms of erythrocytes	81.50±1.93	77.00±0.68*
Discolets	14.00±1.37	18.83±1.08*
Reversibly changed (transitional)	3.33±0.80	3.17±0.48*
Irreversibly changed (Prehemolithic)	1.17±0.48	1.00±0.26*

*$p<0.05$ in comparison with the group of patients having kidney cysts

The study of the morphological state of erythrocytes in the group of patients with kidney cancer (KC) as compared with the group of patients with kidney stones showed that the patients with kidney cancer (KC) had a significant ($p<0.05$) decrease of discocyte number by 5.53%, irreversibly altered (by 4.81%) and degenerative forms (14.53%) of red blood cells. At the same time, there was a significant increase of reversibly altered erythrocytes by 34.5%.

3.2. The study of pathomorphological changes in cells and tissues

The surface of cells is uneven. The structures of irregular shape, revealed in the central deepen-

ing of erythrocytes were of particular interest. The larger size of this structure sides is 0.15 ± 0.03 μm. The plane of these structures has the same globular projections as the rest of the outer contour. Probably, these are the protrusions, which ensure the aggregation of red blood cells in the "coin pillars".

When the external level of the studied blood cells are studied using atomic force microscopy (AFM), it was shown that the depth of a discocyte cavity counted by studying the profile of the cell changed significantly. On average, it made 0.48 ± 0.15 μm, with a malignant course of the disease 0.41 ± 0.03 μm, which was significantly different from the control group (0.38 ± 0.05 μm). With the change in the normal discoidal form on erythrocyte surface larger protrusions were formed along with such structures (Figure 1). The structure of the pores was disturbed.

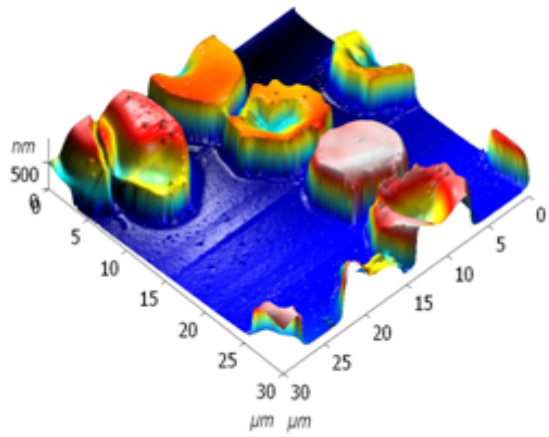

Figure 1. Erythrocytes of blood with diagnosed prostate cancer

The shape and dimensions of the cells are changed. Disturbance of the diameter of the erythrocyte cavity (turquoise color). Change in the structure of the plasma membrane. Individual cells are hemolyzed (pink). Violation of pore structure and cytoplasmic processes on the surface of cells.

Atomic power laboratory. Three-dimensional bar graph.

The following picture was revealed during biopsy study. Thus, during the study of prostate cancer we observed deformed glands with branching structures from which the papillae developed formed by atypical polymorphic cells with weak connections to each other in all cases. A weak connection between the cells is observed easily, which is not determined at the level of light microscopy and indicates the possibility of metastases. It was found that there are more frequent contacts between cells with the value of 0.5 μm and with the progression of the disease, which can reach up to 3-4 μm, exceeding the cell sizes. Besides, the number of tumor cell clones increased on the vascular endothelium (Figure 2). A similar picture of cell cytoarchitecture damage was revealed in PC.

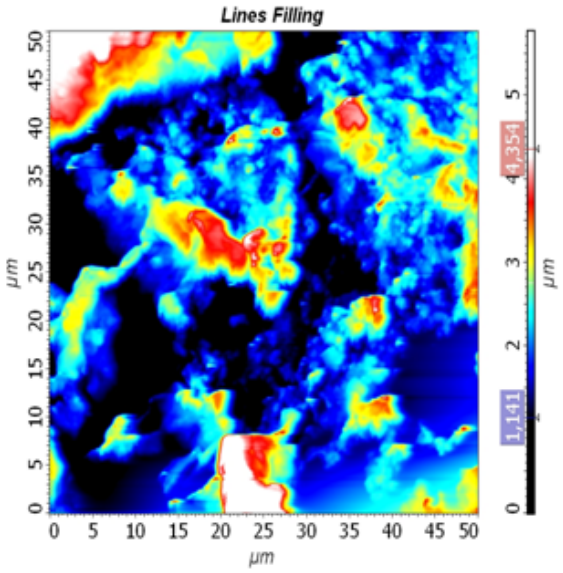

Figure 2. Acinar adenocarcinoma of the prostate gland.

Proliferation of epithelial cells. The cells are not uniform in size and are loosely connected to each other.

Atomic power laboratory. 2D histogram

[IV] SUMMARY:

Thus, using the innovative AFM methods, the data were obtained that indicate a significant change in erythrocyte cytoarchitectonics during cancer processes, especially within a malignant disease.

[V] CONCLUSION:

Thus, the decrease in discocytes was observed after the study, and the number of transitional, pre-hemolytic and degenerative forms of erythrocytes increased. Modified cells are partially functionally inferior and can not fully fulfill their most important function of oxygen transportation.

Pathomorphological changes in the prostate gland revealed deformed glands with branched

structures, from which the papillae developed formed by atypical polymorphic cells with weak connections between each other.

The obtained results broaden the available data and indicate the violation of structural components, which supplements an unfavorable picture of the studied organ functioning. The possibility of the study with atomic force microscopy (AFM) in diagnostic, screening studies and as an express method makes it attractive for pathologists, cytologists and oncologists.

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