Research Article

Methods of scanning blood microscopy in oncology diagnosis of kidney pathology

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ABSTRACT

Background: The purpose of this article is to reveal pathomorphological changes in erythrocytes with oncological processes in the kidneys with the help of atomic force microscopy (AFM) and scanning electron microscopy (SEM). **Methods:** Erythrocytes of patients with kidney pathology are the object of this research. After allocation of erythrocytes, blood samples from a vein were studied in SEMs "FE1 Quanta 200 three-dimensional" and "FE1 Quanta 600 FEG." AFM was done in the probe laboratory Ntegra Aura (Russia). The studies were performed in contact modes of intermittent or constant profiles using commercial Si or SiN cantilevers (NSG01, NT-MDT, Russian Federation) under conditions of low atmospheric vacuum. AFM images were processed and implemented using the NOVA software (NT-MDT, Russian Federation) and image analysis (NT-MDT, Russian Federation). **Results:** The number of transient forms of erythrocytes is increased with aging of patients. There was a decrease of discocytes and an increase of the number of transitional, prehemolytic and degenerative cell forms with kidney cancer. When studying the appearance of erythrocytes with the help of AFM, it was established that the depth of the discocyte cavity counted by studying the cell profile was significantly changing. With the progression of the disease, the cells acquired elongated forms, as well as contacts between the cells, were more common. **Conclusions:** The study has found that the appearance and condition of erythrocyte membranes depend on the age of the recipient and to the greater extent on pathological processes in the kidneys (cysts and cancer) with a change of their cytoarchitecture. The greatest changes have been found in kidney cancer with distant metastases. Modified cells are functionally defective in part and cannot fulfill their most important oxygen transport function in full measure.

KEY WORDS: Atomic force microscopy, Erythrocytes, Kidney cancer, Oncodiagnosis, Scanning electron microscopy

INTRODUCTION

Against the background of the world population growth, the number of oncological pathology is increasing. The urinary system tumor damages are the most urgent problems nowadays.[1-6] Men fall ill about twice as often as women, mostly at the age of 60 years and older. Kidney cancer often appears for the 1st time with metastases without any clinical symptoms of the primary tumor.[1-6] The general clinical manifestations of kidney cancer consist of factors which characterize any blastomatous process and more specific symptoms (polycythemia, sudden arterial hypertension, and fever). Local signs of kidney cancer are declared themselves by a triad: Hematuria, pain in the lumbar region, and a palpable tumor. The prognosis of the disease largely depends on the stage of the tumor and the radical nature of surgical treatment. The overall 5-year survival rate varies from 40% to 50%, in case of localized forms - 75–80%, with the spread of the tumor process - 10–20%.[1-6] Reliable statistics show that the survival of patients with kidney cancer depends on age.[7]

The scientific literature shows that changes in the number of erythrocytes, their size and shape are an important diagnostic criterion in identifying various diseases.

Thus, changes in the erythrocyte membrane were detected in endocrine and metabolic disorders, nervous system damage and mental disorders, digestive system, cardiovascular pathology, respiratory, and endocrine diseases.[8] Changes in the structural and functional properties of erythrocytes were detected when the erythrocytes were involved in a complex set of changes in the tumor process in the human body.

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It was conducted a complex study of erythrocyte damage patterns in oncopathology of lungs, stomach, large intestine, head and neck, and in cancer of ovarian, stomach and bladder cancer, the prostate gland, and thus, it was determined the dependence of the erythrocyte change on the severity of the process.[7,9,10]

The purpose of this work is to reveal pathomorphological changes in erythrocytes with oncological processes in the kidneys with the help of atomic force microscopy (AFM) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Within the framework of this work, 118 patients with kidney diseases were examined and divided into three groups according to a nosological criterion. The 1st group included 40 patients of middle age with kidney cancer with the age range from 40 to 49 years (the average age is 44.3 ± 0.45). Among this group, there were patients who have kidney cancer without metastases (n = 14), those who have kidney cancer with metastases in regional lymph nodes (n = 13) and those who have kidney cancer with distant metastases (n = 13). The 2nd group included 67 elderly patients with kidney cancer whose age range was from 60 to 70 years (the average age is 68.0 ± 0.78). Among this group, there were patients who have kidney cancer without metastases (n = 15), those who have kidney cancer with metastases in regional lymph nodes (n = 20) and those who have kidney cancer with distant metastases (n = 32). The third group included 11 elderly patients with kidney cysts whose age range was from 60 to 78 years (the average age is 61.2 ± 2.4). The control group consisted of 70 practically healthy men and women.

The Study of the Morphological Characteristics of Erythrocytes

The object of the study is the red blood cells of cancer patients: Blood samples taken from a vein were viewed in SEMs "FE1 Quanta 200 three-dimensional" and "FE1 Quanta 600 FEG" after isolation of cells. AFM was produced in the probe laboratory Ntegra Aura (Russia). The studies were performed in contact modes of intermittent or constant profiles using commercial Si or SiN cantilevers (NSG01, NT-MDT, Russian Federation) under conditions of low atmospheric vacuum. The processing and implementation of AFM images were formulated using the NOVA software (NT-MDT, Russian Federation) and image analysis (NT-MDT, Russian Federation).

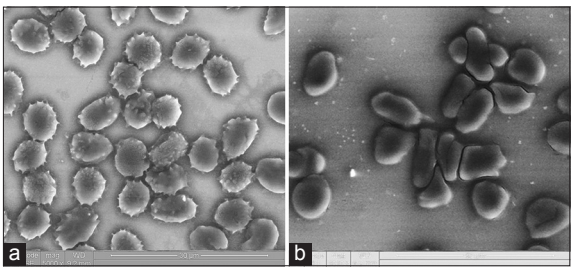

Figure 1: (a and b) Erythrocytes of blood with diagnosed kidney cancer. The shape and dimensions of the red blood cells are changed. The structure of the membrane is broken. Sludge cells. Raster electron microscopy (× 5000)

RESULTS AND DISCUSSION

The Study of Morphofunctional Characteristics of Erythrocytes in a Group of Practically Healthy People

Morphofunctional characteristics of erythrocytes in a group of practically healthy middle-aged people

The analysis of the obtained data of SEM in a group of practically healthy middle-aged people reflected the ratio of the sizes of erythrocytes. Thus, a large percentage of the erythrocyte population was represented by normocytes - 83.3 ± 1.43%, the share of microcytics accounted for 16.3 ± 1.52%, and macrocells were 0.4 ± 0.14%. The average erythrocyte size in the control group was 7.10 ± 0.04 μm. The diameter of microcytes was 6.1 ± 0.04 μm, the normocytes were 7.2 ± 0.04 μm, and the average size of macrocytes was 9.2 ± 0.07 μm. The distribution of erythrocytes depending on their size had the form of a curve relative to the regular shape with a base lying in the range of 5.20–9.30 μm. The peak of the graph corresponded to the point of 7.1 μm.

In the control group of middle-aged patients, the prevalence of discocytes was 88.67 ± 2.44%. The content of transitional forms which are able to reverse transformation (ellipses, discocytes with a crest, flat discs, discocytes with an outgrowth, discocytes with multiple outgrowths, and erythrocytes in the form of a "mulberry") amounted 11.0 ± 2.39% and prehemolytic (irreversible) forms (domed, spherical, and in the form of "deflated ball") - 0.17 ± 0.17% and the same number of degenerative ones.

Morphofunctional characteristics of erythrocytes in a group of practically healthy elderly people

The study of erythrocytes in a group of practically healthy elderly people showed that the prevalent percentage of red blood cells was in normocytes (Figure 1).

The content of normocytes in the elderly age is lower than in the middle-aged group (75.6 ± 3.14%) and the number of microcytes is significantly increased (23.3 ± 3.28%). Macrocytes were only in 1.1 ± 0.28% of the total number of scanned cells. Cytometric analysis showed that the average size of the erythrocytes was 7.00 ± 0.05 μm. The diameter of microcytes in the elderly age was 5.9 ± 0.06 μm that is significantly lower than in the control group of middle-aged patients (P < 0.05). The diameter of normocytes was 7.3 ± 0.04

μm, and the average size of macrocytes was 9.2 ± 0.07 μm. The size distribution curve had a base range of 4.50–9.29 μm and a vertex corresponding to a point of 7.0 μm for erythrocytes in elderly age.

The study results of the erythrocytes morphological characteristics in elderly people showed the following ratio of the main forms: Discocytes - 86.67 ± 0.80% [Figure 2]; transitional forms - 10.00 ± 0.52%; prehemolytic ones - 3.00 ± 0.37%; and degenerative forms of erythrocytes - 0.33 ± 0.21%. There was a significant ($P < 0.05$) increase in the percentage (3.00 ± 0.37%) of irreversibly altered erythrocytes in comparison with the group of practically healthy middle-aged people. Furthermore, in this group, there were single dome-shaped erythrocytes and erythrocytes in the form of a "deflated ball."

Morphofunctional Characteristic of Erythrocytes in a Group of Patients with Renal Pathology of the Elderly Age

Morphofunctional features of erythrocytes in a group of elderly aged patients with kidney cysts

The study of blood samples of elderly patients with kidney cysts revealed a morphological heterogeneity of erythrocyte mass. The content of normocytes in the group was 91.9 ± 1.08%. A significant ($P < 0.05$) decrease in the percentage of microcytes (0.4 ± 0.14%) was revealed in comparison with all studying groups. There was also an increase in the macrocyte content-7.8 ± 0.95% ($P < 0.05$) in comparison with the control groups of both ages. Cytometric data showed that the average size of red blood cells is 7.90 ± 0.05 μm. The diameter of the microcytium (6.4 ± 0.07 μm) is greater in relation to all study groups (significant [$P < 0.05$] to groups of practically healthy people of both ages). The diameter of normocytes (7.8 ± 0.04 μm) is more significantly ($P < 0.05$) in comparison with all studying groups. The average size of macrocycles is 9.5 ± 0.08 μm that is significantly higher than this indicator in control groups of both ages. The graphic distribution of erythrocytes in size was in the form of a curve which base (6.40–10.20 μm) and vertex (7.9 μm) were sharply shifted to the right.

The study results of the shape and relief of the erythrocyte surface in elderly patients with kidney cysts revealed that the number of discs was 81.50 ± 1.93% that is significantly ($P < 0.05$) lower in comparison with control groups of both ages, and significantly higher relatively to the group of elderly patients with kidney cancer ($P < 0.05$). The content of transient forms of erythrocytes (14.00 ± 1.37%) is significantly ($P < 0.05$) higher in comparison with the group of practically healthy elderly people, and significantly ($P < 0.05$) lower in relation to elderly patients suffering from malignant neoplasms of kidney. Prehemolytic forms were 3.33 ± 0.80% and degenerative ones were 1.17 ± 0.48% [Table 1].

Morphofunctional characteristics of erythrocytes in a group of elderly aged patients with kidney cancer

The results of hemoscanning showed that in elderly people with diagnosed kidney cancer the main part of erythrocytes is represented by normocytes 93.3 ± 0.47%. At the same time, this indicator is higher among the studying groups and the content of microcytes (5.2 ± 0.34%) in the erythrocyte population was significantly lower in comparison with the control group and higher relatively to the group of patients with kidney cysts ($P < 0.05$). Macrocytes accounted for 1.5 ± 0.27%. The diameter of macrocytes was 9.7 ± 0.19 μm that is significantly ($P < 0.05$) higher than in the group of elderly patients with kidney cysts. In this group, the average size of red blood cells was 7.40 ± 0.04 μm. The diameter of microcytes was 6.2 ± 0.05 μm that is significantly ($P < 0.05$) higher than in the control group. The diameter of normocytes is 7.4 ± 0.03 μm that is significantly ($P < 0.05$) higher than in control groups of both ages and also significantly ($P < 0.05$) lower in comparison with the group of elderly patients suffering from the kidney cyst [Tables 1 and 2]. The graphical recording of the distribution of erythrocytes in terms of magnitude was represented by a curve with a wide foundation in the range of 5.90–10.10 μm and a vertex at a point equal to 7.4 μm. The depression on the cell surface is smoothed.

The distribution of erythrocytes in elderly patients with kidney cancer by main groups was such that

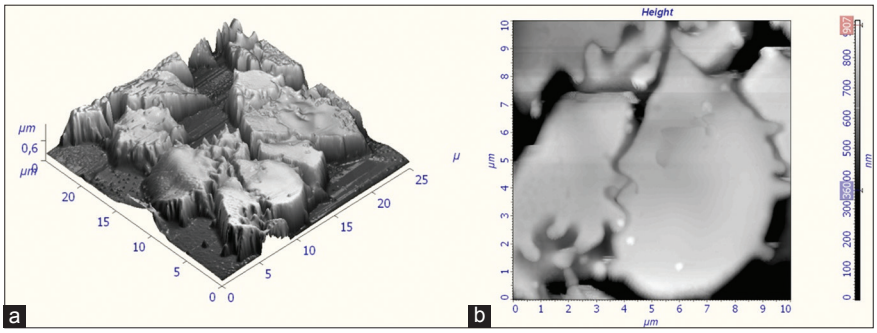

Figure 2: The shape and dimensions of the red blood cells are changed. The structure of the membrane is broken. Sludge cells. Atomic power laboratory. (a) Three-dimensional bar graph, (b) two-dimensional bar graph

Table 1: The ratio of morphological forms of erythrocytes in elderly patients with kidney pathology

Morphological forms of erythrocytes	A group of elderly patients with kidney cysts	A group of elderly patients with kidney cancer
Discolets	81.50±1.93*.**	77.00±0.68**
Reversibly changed (transitional)	14.00±1.37**,xx	18.83±1.08*,**,x
Irreversibly changed (prehemolytic)	3.33±0.80*	3.17±0.48*
Degenerative forms	1.17±0.48	1.00±0.26*

Annotation: *P<0.05 in comparison with the group of practically healthy middle-aged people; **P<0.05 in comparison with a group of practically healthy elderly aged people; xP<0.05 in comparison with a group of elderly patients with kidney cysts; xx P<0.05 in comparison with a group of elderly patients with kidney cancer

Table 2: Characteristics of erythrocyte sizes in elderly patients with pathology of the kidneys

The ratio of erythrocyte sizes		
The ratio of erythrocyte sizes	**A group of elderly patients with kidney cysts**	**A group of elderly patients with kidney cancer**
Microcytes (%)	0.4±0.14*.**,xx	5.2±0.34**,x
Normocytes (%)	91.9±1.08	93.3±0.47
Macrocytes (%)	7.8±0.95*.**,xx	1.5±0.27x
Dimensions of erythrocytes		
Size of erythrocytes	**A group of elderly patients with kidney cysts**	**A group of elderly patients with kidney cancer**
Diameter of erythrocytes (μм)	6.80±0.06	7.90±0.05
Diameter of microcytes (μм)	6.4±0.07*.**	6.2±0.05**
Diameter normocytes (μм)	7.8±0.04*.**xx	7.4±0.03*.**,x
Diameter macrocytes (μм)	9.5±0.08*.**	9.7±0.19

Annotation: *P<0.05 in comparison with the group of practically healthy middle-aged people; **P<0.05 in comparison with a group of practically healthy elderly aged people; xP<0.05 in comparison with a group of elderly patients with kidney cysts; xx$P$<0.05 in comparison with a group of elderly patients with kidney cancers

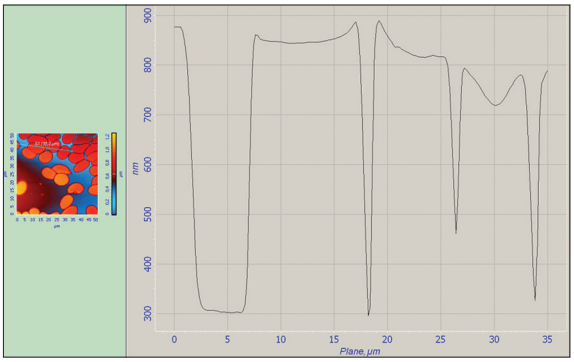

Figure 3: The cell sizes are different. The outer cavity in the part of the cells is smoothed. Atomic power laboratory. Bar chart

the percentage of discocytes accounted for 77.00 ± 0.68% that is significantly (P < 0.05) lower than in the control group. The content of transient forms of erythrocytes (18.83 ± 1.08%) is significantly higher than in groups of practically healthy patients of both ages and in groups of patients with kidney cysts (P < 0.05). An increase in the content of transient groups of erythrocytes was due to a significant increase (P < 0.05) in the discocyte content with an outgrowth (4.33 ± 0.61%), ellipses (2.33 ± 0.42%), and discocytes with multiple outgrowths (10.83 ± 0.79%) in comparison with all the studying groups. The content of prehemolytic forms (3.17 ± 0.48%) was relatively (P < 0.05) higher in comparison with the group of practically healthy middle-aged people. In this group, there were single dome-shaped red blood cells and erythrocytes in the form of a "deflated ball." The content of degenerative forms was 1.00 ± 0.26% that is significantly (P < 0.05) higher in comparison with the group of practically healthy middle-aged people. The cavity on the surface of cells is smoothed even more and erythrocytes acquire an elongated shape (Figure 3).

CONCLUSIONS

Thus, as a result of the study, it was revealed that the appearance and condition of the erythrocyte membranes depends on both the age of the recipients and, to a greater extent, on the pathological processes in the kidneys (cysts and cancer) with a change in their cytoarchitectonics. The greatest changes were found in kidney cancer with distant metastases. The modified cells are functionally inferior in part and cannot fulfill their most important oxygen transport function in full measure. The obtained results broaden the available data and indicate a violation of the structural components of the blood that is the supplement to the unfavorable picture of the organism functioning. The possibility of research using SEM and AFM in diagnostic, screening studies and as an express method makes it attractive for pathologists, cytologists, and oncologists.

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