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THE DYNAMICS OF THE CELLULAR COMPOSITION OF URINE SEDIMENT IN CONDITIONS OF AN ARTIFICIAL BLADDER

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Abstract

Aim. The evaluation of the urine cytological profile in the dynamics of adaptation of the orthotopic bladder to the functional properties of the urinary reservoir and determination of the connection between the cellular composition of the urine with the functional role of the formed from the intestinal fragment neocyst in experimental conditions and in humans.

Materials and methods. The material in experimental study were the results of a cytological study of the urine sediment obtained from 21 female pigs (mini-pigs). The portion of urine had received in experimental animals one month after orthotopic ileocystoplasty, 6 months and 1 year later and prepared as smear from the resulting precipitate. The level of lymphocytes, neutrophils, erythrocytes, as well as the superficial, intermediate and basal epitheliocytes evaluated during study. An analysis of the cell composition of centrifugal smears from the neocyst were performed in 21 patients with bladder cancer undergoing cystectomy and the formation of an orthotopic bladder, at various periods after surgery, from 3 months to 5 years. The preparation of a centrifugal sediment and the subsequent production of smears carried out as well as experimental animals.

Results and conclusions. The results of study showed that the formation of an artificial bladder in experimental animals with a fragment of the ileum changes the composition of the cells of the urinary sediment mucosa. These changes consist of an increase in the content of red blood cells, a decrease in the number of lymphocytes and neutrophils and change in the number of epithelial cells. The cellular composition of the sediment changes with the increasing duration of the postoperative period and manifests by reduction and even disappearance of erythrocytes in smears. The level of lymphocytes and neutrophils is close to those observed in intact animals. The content and ratio of epithelial cells also approached to experimental animals. The structure of the organ and its function are closely related and there can be the adaptation of the tissues of the intestinal wall to new conditions. It may have accompanied the topography of the location of the vessels, which reduces the likelihood diapedetic yield of erythrocytes. It can be assumed that the lymphoid follicles of the mucosa of the intestinal wall are atrophied and the mucosal protection approaches that observed in the intact bladder. It is also possible to transform the epithelium of the mucosa, which changes the composition of epithelial cells in the sediment. The study of the urine sediment centrifuge in the dynamics of functioning of neobladder formed from the ileum wall showed similar dynamics of its cellular composition. The same adaptive adjustment processes take place as in the experimental animals. One of the factors of oncogenesis is the disregulatory pathology of regeneration processes. It allows to establish that a regular assessment of the composition of urine centrifuge cells will allow diagnosing possible shifts in the regulation of regeneration processes in patients after formation of artificial bladder. It is necessary for oncological control of local recurrence and early detection of urothelial cancers of the upper urinary ways.

Key words: bladder cancer, neobladder, ileum.

Bladder cancer is the most common malignant disease of the urinary tract, with a peak incidence in adults and the elderly [1]. The creation of an artificial bladder with the formation of an orthotopic conduit has become a standard treatment for invasive bladder cancer [2, 3, 4]. The ileum is preferable for the formation of urinary reservoirs due to the least reabsorption of electrolytes [5, 6, 7, 8, 9]. The ileum intestine, less often thick, and even less often stomach [10] are used as neocyst. A segment of the ileum is isolated to form a reservoir from the gastrointestinal tract. The latter is detubulated and then stitched together in the form of S, W, U, M, N - anastomoses, turning into the capacity of the shape of the ball [11, 12].

Academician I.V. Davydovsky formulated the basic thesis of functional morphology: "There is no change in structure without changing the function and changing the function

without changing the structure" [13]. It is logical to assume that changes in the function of the ileal part during the formation of the neobladder, in contact with the urine and the performance of new functions should influence on the ultrastructure of its wall. However, any available data on cytological monitoring of possible changes and indications of the criteria by which this monitoring could be performed were absent in available literature.

The cellular material in the form of desquamated epithelial cells located on the surface, passes during functioning from the surface of any mucous membrane to the adjacent liquid medium. The change in the structural and functional organization of the neobladder mucosa should influence the composition of cells and the desquamation of the epithelium.

The aim of study is the evaluation of the urine cytological profile in the dynamics of adaptation of the orthotopic bladder to the functional properties of the urinary reservoir and determination of the connection between the cellular composition of the urine with the functional role of the formed from the intestinal fragment neocyst.

Materials and methods

The material of the experimental renewal of the cellular transformations of the intestinal mucosa were the results of a cytological study of the urinary sediment obtained from 21 female-pigs (mini-pigs), which modeled the neobladder from the part of the ileum. Scientists have proved that the organism of pigs, according to its anatomical and morpho-functional features, is closest to human and ideally suited for studying the pathogenesis of various morphological and biochemical processes. The ratio of the age of pigs to humans is generally considered to be 5: 1 [14]. Neocyst formation was performed by extirpation of the bladder in endotracheal anesthesia. The formation of conduit from the part of the ileum, was performed 10 cm from the ileocecal angle, then a segment of 10 cm was separated, dissected into an anti-brazier margin and formed a ball shape. The ureters were implanted in the top of the conduit, and the lower end was anastomosed with the urethra.

A urine sample from experimental animals received by catheterization one month after the operation, 6 months and 1 year later was centrifuged at 3000 rpm for 15 minutes. The smears prepared from the resulting precipitate were air dried, subsequently fixed in alcohol ether and stained with hematoxylin-eosin. Subsequently, the resulting preparations were examined with a light microscope. The five fields of vision were counted (in total not less than 150 cells) in each preparation. The level of lymphocytes, neutrophils, erythrocytes, as well as the superficial, intermediate and basal epitheliocytes evaluated during study. The evaluation of the level of epithelial cells was carried out proceeding from the notion that the transformation of the intestinal wall should be accompanied by a structural and functional

rearrangement of the epithelium. The data obtained in the study of the cytological picture of swabs of the ileum of the same pigs were used as a control. It was obtained before the operation of the bladder replacement with an intestinal fragment, treating them as intact.

The analysis also carried out the cellular composition of neocyst centrifuge smears. The cystectomy in 21 patients with the formation of an orthotopic urinary bladder in different periods was made to get the material. The periods of surgery were from 3 months to 5 years. The preparation of a centrifugal deposit and the subsequent production of smears was performed as well as experimental animals. The results of the research showed in relative figures and summarized in a table.

The animals were taken out of the experiment by decapitation during anesthesia, adhering to the provisions of the "European Convention for the Protection of Vertebrates used for experiments or for other scientific purposes" (Strasbourg, 1986).

The reliability of the discrepancy (P) between the values was determined by the Student's t test. Differences were considered statistically significant at $p < 0.05$.

Results and its discussion

The study results of smears of experimental animals are set out in Table.1.

Tab. 1 Dynamics of the cellular composition of urine sediment in neocyst modeling in experimental animals (n = 21)

Group / Indicator %	Control (M ± m)	1 month (M±m)	6 months (M±m)	12 months (M±m)
Lymphocytes	7,5±0,3%	5,0±0,8%*	3,9±0,3%*	9,0±0,9%*
Neutrophils	18,2±0,5%	6,6±0,8%*	12,4±0,7%*	17,2±0,9%*
Erythrocytes	0,4±0,1%	50±2,1%*	23,2±1,4%*	19,0±1,1%*
Epithelium basal	24,7±3,9%	28,4±2,2%	27,9±0,7%	16,5±0,6%*
Epithelium superficial	48,3±2,8%	10±2,1%*	28,7±3,5%*	34,7±2,9%*
Other types of epithelium	0,9±0,1%	–	3,9±0,1%*	2,6±0,3%*

The neutrophils and lymphocytes presented in a sufficiently large amount in the urinary centrifugate smear of intact animals (Table 1). The presence of these cells indicates a pronounced local immune defense, obviously, to ensure the sterility of the bladder cavity.

The ratio of cells in the newly created bladder was different from normal, namely, the lymphocyte count was reduced by 33.3% and neutrophils by 63.7%, the percentage of red blood cells increased almost 50 times, the proportion of epithelial cells decreased by 79.3% after the operation, after 1 month, as can be seen from Table 1. This situation is connected, with the fact that the mucosa of the neocyst formed from the intestine changes by contact with

the urine. The appearance of a large number of erythrocytes is the result of a microtraumatic mucosa. There are many lymphoid follicles, which protect the mucosa without creating a large concentration of lymphocytes and neutrophils on the surface in the submucosa. Simultaneously, the state of the epithelial lining of the ileum should be indicated, which is represented by a prismatic epithelium. It could be due to mechanical trauma, and not due to natural renewal. A sharp decrease in the surface epithelium by 79.3% is noteworthy, and the basal epithelium does not significantly change in animals after observations at 1 and 6 months.

The level of lymphocytes stabilized and even increased by 16.7% compared with the control at the end of the experiment. The level of neutrophils was approached the control data. It can be assumed that during this period in the transformation of the artificial bladder wall occurs the submucosal follicles degrade, and the mucosal protection passes into a regime characteristic for a typical mucosa of the bladder. At the same time, the proportion of red blood cells decreases by 62% compared to the data in animals after 1 month. Apparently, it can be due to the coarsening of the urinary conduit wall and adaptation to traumatization. The composition of the epitheliocytes of the centrifuge approximates the composition of the intact animals. The amount of superficial epithelium increases by 71.2% after 12 months in comparison with the indices in animals after 1 month, but still reduced by 28.2% compared to the control. The prismatic epithelium is transformed and the renewed epithelium acquired the properties, shape and appearance of the urothelium. However, this process takes a long time, as evidenced by the appearance of non-standard epitheliocytes.

The change in the cellular composition of the urinary sediment centrifuge in persons with enterocystoplasty after cystectomy for bladder cancer at different times after the operation were also investigated in study. The results of these studies are shown in Table 2. The data in Table 2 shown the composition of the cells of the ileum wash centrifuges in healthy people is not fundamentally different from the data in experimental animals. The existing quantitative differences between the boundaries of physiological corridors are not fundamentally different.

Analysis of the dynamics of the urine centrifuge cellular composition in persons with neocyst suggests the following; the lymphocyte content increased by 2.5 times, and the neutrophils decreased by 33% during the first year. These indicators are close to those observed in the control group two years after the operation. An involution of the intestinal lymphatic follicles is occurred during this period and the immune defense system of the mucosa approaches the usual for the bladder.

Tab. 2 Dynamics of the cellular composition of a urine centrifuge in patients with an artificial bladder (n = 21)

Group / Indicator %	Control (M±m)	1 year after operation (M±m) (n=21)	2 years after operation (M±m) (n=17)	3 years after operation (M±m) (n=14)
Lymphocytes	8,80±0,5%	21,4±0,6%*	14,2±0,3%*	27,9±0,7%*
Neutrophils	18,5±2,4%	12,4±1,4%*	14,6±1,6%*	17,7±1,9%
Erythrocytes	0,5±0,02%	41±1,2%*	5,7±0,2%*	-
Epithelium basal	27,8±1,7%	26,8±1,1%	18,0±2,1%*	17,7±1,4%*
Epithelium superficial	43,8±1,1%	10,8±2,3%*	47,5±3,5%	36,7±2,8%*
Other types of epithelium	0,6±0,1%	-	-	-

Simultaneously, the red blood cells decrease and disappear in the centrifuge against the background a sharp increase of erythrocytes in 82 times in the urine sediment a year later and two years later. This may be due to the thickening of the wall of the neocyst, the condensation of the vessels in it, and, correspondingly, to a decrease in the possibility of diapedesis of the erythrocytes into the neocyst cavity.

The content of epithelial cells, their ratio over the course of 3 years acquires a parity characteristic of transitional epithelium. A sharp increase in the proportion of cells that are similar in structure to the basal cells of the transitional epithelium was at the 3 year of observation. It may be due to the transformation of the epithelial cover or change in the process of regeneration of the epithelium. The latter variant requires careful study, as oncogenesis is largely associated with dysregulation.

Thus, the results of our studies showed that the formation of an artificial bladder in experimental animals with a fragment of the ileum changes the composition of the cells of the urinary sediment mucosa. These changes consist of an increase in the content of red blood cells, a decrease in the number of lymphocytes and neutrophils, change in the number of epithelial cells. The cellular composition of the sediment changes with increasing duration of the postoperative period. It is manifested by the decrease and even disappearance of erythrocytes in smears. The level of lymphocytes and neutrophils is close to those observed in intact animals.

The content and ratio of epithelial cells also approaches to intact animals. There is adaptation of the tissues of the intestinal wall to new conditions. It is accompanied by topography of the vascular arrangement, which reduces the likelihood of diapedesis of erythrocyte output. The lymphoid follicles of the mucosal intestinal wall are atrophied and the

mucosal protection approaches to that observed in the intact bladder. It is also possible transformation of the mucosal epithelium, which changes the composition of epithelial cells in the sediment.

The study of the urine sediment centrifuge in patients during the dynamics of functioning formed from the ileum wall neobladder showed similar dynamics of its cellular composition. The same adaptive adjustment processes take place as in the experimental animals.

One of the oncogenesis factors is the disregulatory pathology of regeneration processes. A regular assessment of the composition of urine centrifuge cells will allow to diagnose possible shifts in the regulation of regeneration processes in patients after the formation of artificial bladder and necessary for oncological control of local recurrence and early detection of urothelial cancers of the upper urinary ways.

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