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INDICATORS OF EXPRESSION LEVEL OF Bcl-2 PROTEIN IN BLADDER LEUKOPLACIA

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Annotation
In this work, indicators of the level of expression of the anti-apoptotic protein Bcl-2 in different stages of bladder leukoplakia were determined. The results showed that in the control group, this protein was expressed at a low level only in the basal layer of the lining epithelium of the urinary bladder. During the initial stage I of the development of leukoplakia, during the emergence of the metaplastic process in the changing epithelium, the expression of Bcl-2 protein was observed to increase to a high level in the cells of the basal layer of the epithelium with acanthosis. In the II stage of leukoplakia, all cells of the epithelial layers are metaplastic and located vertically, Bcl-2 protein is expressed at a relatively higher level in the cells of the basal and intermediate layers, and in the III stage of the disease, the expression of this protein is even stronger.

Keywords: bladder, cystitis, leukoplakia, immunohistochemistry, Bcl-2 protein.

The Urgency of the Problem

The 6th protein of the Bcl-2 domain, which is located on human chromosome 18 and has anti-apoptotic properties out of 16 proteins, is a homologous protein that slows down the process of apoptosis. This protein with a molecular weight of 22 kDa is located in the cell and nuclear membrane, sarcoplasm and mitochondrial membrane [2, 4, 7]. Hyperexpression of this protein stops the release of calcium ions and slows down lipoperoxidation, inhibits antioxidant activity, and slows down the activity of NO-synthetase. The main function of Bcl-2 is to stop cytochrome S, AIF, ATF, which are anti-apoptosis molecules, from the mitochondria. Bcl-2 binds to the mitochondrial membrane and closes pores, stops proapoptotic signals and prevents apoptosis [1, 3, 8].

Leukoplakia of the urinary bladder can develop under the influence of various pathological factors, as a result of which the cells of the covering epithelium of the urinary bladder are dead due to the process of programmed apoptosis. But in most cases, due to chronic diseases, the apoptosis process of cells can slow down and stop. Therefore, in our study, we aimed to study the anti-apoptotic protein Bcl-2 in the lining epithelial cells in bladder leukoplakia. Increased Bcl-2 activity is observed in a number of bladder diseases, including leukoplakia. Due to the development of inflammatory and disregenerative processes in the submucous connective tissue layer of the bladder leukoplakia, the differentiation of cells in the covering epithelium is disturbed and often lags behind, the proliferative activity of the cells of the basal layer increases, and the anti-apoptotic protein Bcl-2 can be activated in them. [2, 5, 6]. The expression level was evaluated as the percentage of stained cells (x400) from the field of view. The spread and intensity of the immunohistochemical reaction in the sample was taken into account, i.e. (if there is no expression or if less than 10% of cells are stained -0 points, from 10 to -25% -1 point, from 26 to -50% -2 points, from 51 to -75% -3 points and more than 75% is -4 points.

Material and Methods

As material, 108 patients living in Fergana region, with a history of chronic cystitis (48.2%), bladder neck cystitis (36.0%), urethral cystitis (14.0%), cystalgia (24.6%) a biopsy of the mucous membrane of the urinary bladder obtained by cystoscopy was taken from women. The age of patients is from 18 to 84 years, the average age is 32.6 years. The duration of their illnesses was 6 months to 8 years, on average 2.7±0.9. Biopsies were frozen in 10% neutral formalin for 48 hours. Dehydration was carried out in alcohols and chloroform of increasing concentration. Histological sections were initially stained with hematoxylin and eosin to determine their topography. Then, a series of sections obtained from paraffin blocks were deparaffinized, dehydrated, unmasked, and stained for antigens in a specially automated Ventana Benchmark XT system, Roche, Switzerland. Vcl2 was detected using antibodies.

Research Results and Their Discussion

In order to determine the pathomorphological and immunohistochemical changes occurring in the covering epithelium of the bladder, biopsy material obtained from people without any pathology in the bladder was studied as a control group. Then, the pathomorphological and immunohistochemical changes in the mucous membrane and covering epithelium of the urinary bladder were studied in comparison with each

other according to the clinical-morphological forms and development periods of leukoplakia.

In the control group, it was found that the epithelium covering the mucous membrane of the urinary bladder in patients consists of the usual multi-layered variable epithelium, and its epithelial cells located in the basal layer are relatively large, hyperchromic, arranged in the basement membrane, and most of their nuclei are elongated and elongated. In the surface layers of the multi-layered epithelium, it was observed that the cells are relatively rare, their nuclei are smaller in size, their color is lighter, and their location is flattened. The results of the immunohistochemical examination for the detection of epithelial cell anti-apoptosis protein showed that in the control group, this protein was expressed at a very low level in the cytoplasm of some relatively young and cambial cells located only in the basement membrane, and it was not expressed in other cells of the intermediate and surface layers (Figure 1).

The next task of the research was to study the level of expression of the anti-apoptotic protein Bcl-2 as the proliferative activity of epithelial cells increased in different periods of leukoplakia, and the following data were revealed. The initial period of leukoplakia development is determined by the appearance of metaplastic processes in the changing epithelium. In this case, the number of multi-layered epithelial layers increases, the epithelium of the surface layers flattens, and due to the increase in the amount of glycogen and prokeratin in the cytoplasm of the cells, it takes on a bubble-like appearance. As a result of immunohistochemical examination, due to the increased proliferative activity of the multi-layered variable epithelium of the urinary bladder, it was observed that strong acanthosis developed in the basal part, and the connective tissue layer under the basal layer epithelium grew in bundles.

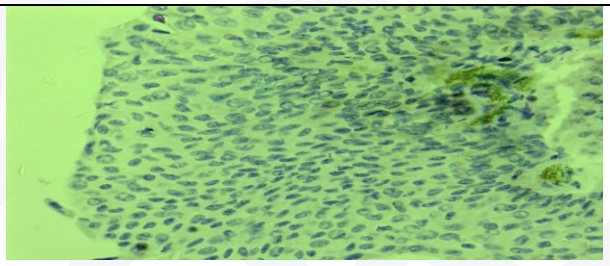

Figure 1. Bladder, norm, Bcl-2 protein is expressed at a low level in some cells of the basal layer. Staining: immunohistochemistry. Floor: 10x40.

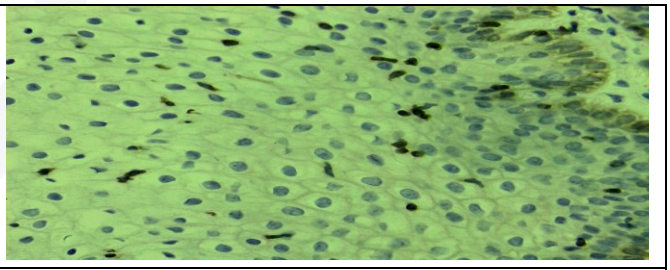

Figure 2. Bladder, leukoplakia grade I, Bcl-2 protein is expressed in some cells of the basal layer and intermediate layer. Dye: immunohistochemistry. Floor: 10x40.

When multi-layered variable epithelia were viewed in general, it was observed that Bcl-2 was expressed at a low level in the 1st row of cells of the basal layer, in the form of light brown cytoplasmic inclusions (Fig. 2). As the cytoplasm of the cells of the middle and surface layers of the multi-layered epithelium underwent hydropic dystrophy and became vacuolated, it was observed that Bcl-2 protein began to be

expressed locally in some of them. In the first period of leukoplakia, the epithelium covering the mucous membrane of the urinary bladder was studied under the microscope lens, and it was found that, except for the epithelium of the basal layer, almost all the epithelium of the middle and superficial layers underwent metaplasia, that is, they became flattened, hydropic dystrophy, and became vacuolated. As a result, it was found that Bcl-2 appeared in the nuclear membrane in the cytoplasm of some of the metaplastic cells, and the brown insert was dense in the nucleus (Fig. 3). Quantitatively, Bcl-2 marker expression was found to be 2 points, i.e., staining occurred in 19.3% of cells. This morphological and immunohistochemical condition showed that epithelial cells proliferated and produced anti-apoptosis protein.

Figure 3. Bladder, leukoplakia grade I, Bcl-2 protein is expressed close to the nucleus of interstitial epithelial cells. Staining: immunohistochemistry. Floor: 10x100.

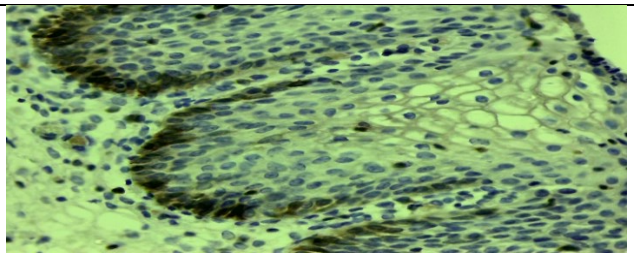

Figure 4. Urinary bladder, leukoplakia grade II, Bcl-2 is expressed in the basal layer 2-3 rows. Staining: immunohistochemistry. Floor: 10x40.

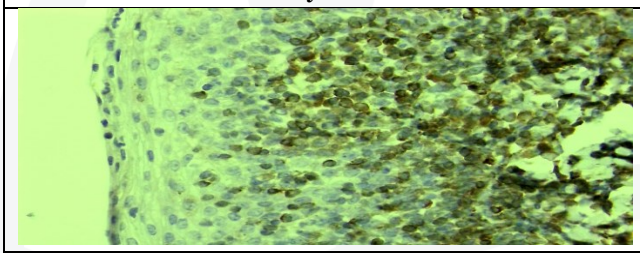

Figure 5. Bladder, leukoplakia grade III, Bcl-2 was expressed in most cells of the basal and intermediate layers. Staining: immunohistochemistry. Floor: 10x40.

During the II stage of leukoplakia of the lining epithelium of the urinary bladder, that is, during the change in the appearance and shape of the epithelial cells, it was found that the changing epithelium turned into a multi-layered flat epithelium and almost all of its cells were located vertically. It was observed that the cells of the basal layer consist of relatively small and darkly stained epithelium, and the cells of the surface layer are relatively large and swollen and enlarged due to the increase of keratohyalin in their cytoplasm. The immunohistochemical examination of this II-period of the disease showed that, unlike the I-period, Bcl-2 protein was expressed in the cytoplasm of some of the cells of the 2-3 rows and intermediate layers located in the basal layer in a dark brown color (Figure 4). This situation indicates that during the II period of

leukoplakia, the proliferative activity of the epithelium of the basal and intermediate layers increased, Bcl-2 protein stuck to the mitochondrial membrane and closed the pores, interrupted the proapoptotic signals and stopped the development of apoptosis. As mentioned above, in this period of the disease, the multi-layered changing epithelium is oval and elongated and is located vertically. Bcl-2 positively expressed interstitial cells were elongated in shape, and their cytoplasm was relatively narrow and light brown in color. Quantitatively, it was detected at the level of 3 points, that is, in 37.3% of cells.

The difference between the III stage of leukoplakia of the multi-layered changing epithelium covering the mucous membrane of the urinary bladder and the previous stages is that the proliferative activity and metaplasia process has developed in almost all the basal and intermediate rows of the epithelium. Another characteristic feature of this period is the presence of chronic inflammatory infiltrate in the sub-epithelial connective tissue plate, the proliferation of blood vessel wall cells, and the positive expression of Bcl-2 protein in them. In immunohistochemical examination, it is observed that Bcl-2 protein is attached to the nucleus of epithelial cells, expressed in a brown color in the cytoplasm and in the outer cytolemma, this condition was evaluated with a score of 3.5 (43.7%). It is noteworthy that during this period it is confirmed that it is expressed only in the nuclear membrane of some cells of the intermediate layers, and in other cells only in the outer cytolemma.

Conclusion

Immunohistochemical study of bladder leukoplakia, i.e., determining in which layers of the covering multi-layered variable epithelium the anti-apoptotic Bcl-2 protein is expressed, is an important factor in the diagnosis of this disease.

In the control group without any disease in the bladder, Bcl-2 protein is expressed at a low level only in the basal layer, which indicates that the apoptosis activity is preserved in them.

During the initial stage I of the development of leukoplakia, during the emergence of the metaplastic process in the changing epithelium, the expression of the Bcl-2 protein in the cells of the basal layer of the epithelium with developed acanthosis indicates the activation of the anti-apoptotic gene.

It was found that in the II-period of leukoplakia, all layer cells of the epithelium undergo metaplasia and are located vertically, Bcl-2 protein is expressed at a relatively higher level in the cells of their basal and intermediate layers.

During the III stage of leukoplakia, proliferative activity and metaplasia are developed in the cells of all layers of the epithelium, there is inflammation in the private plate, Bcl-2 protein is expressed at a high level in all epithelial cells.

Bcl-2 protein expression increases from immunohistochemical markers in different stages of bladder leukoplakia, i.e., from 19.3% to 43.7% (3.5 points) as the disease progresses. It is recommended to use such numbers as an indicator of predicting the consequences of the disease.

References

1. Al-Shukri, S.Kh. General principles of treatment of patients with bladder cancer. The value of clinical, histological and biological prognostic factors for the choice of treatment / S.Kh. Al-Shukri, I.A. Korneev // Practical oncology. 2004. - T. 4. - No. 4. - S. 204-211.
2. Zabolotneva A.A., Gaifullin N.M., Buzdin A.A., Alekseev B.Ya., Andreeva Yu.Yu., Shegai P.V., Sokov D.G., Rusakov I.G. Molecular markers of bladder cancer from particular to whole. Oncourology. 2011. No. 3. C. 16-19
3. Mager, V.O. Prognostic value of biological markers in patients with superficial and invasive bladder cancer / V.O. Mager, N.V. Kazantseva // Oncourology. - 2006. - No. 4. - P. 30-34.
4. Matveev B.P. Clinical oncourology Verdana. - M. - 2003. - S. 197-270.
5. Tumor markers in screening and monitoring of patients with bladder cancer / S.P. Darenko, D.V. Perlin, V.N. Parshina, I.V. Chernyaev // Oncourology. - 2005. - No. 3. - S. 51-54.
6. Prognostic factor in transitional cell cancer of the bladder: an emerging role for bcl-2 and p53 / F. Ong, I.V. Moonen, M.P. Gallee et al. // Radioter Oncol. - 2001. - 61(2). - P. 169-175.
7. The role of bcl-2, p53 and Ki-67 index in predicting tumor recurrens for lov grade superficial transitional cell bladder carcinoma / T.T. Wu, Y.H. Chen, Y.Y. Lee Y.R. Huang // Y. Urol. -200. - 163(3). - P. 758-760.
8.McKenney JK. Precursor lesions of the urinary bladder. Histopathology. 2019; 74:68-76.
9. Keshtkar A., Salehnia Z., Keshtkar A., Shokouhi B. Bladder Cancer Detection Using Electrical Impedance Technique (Tabriz Mark 1)// Patholog Res Int. 2012;2012:470101.
10.Huan Wang., Tie Chong., Xiu-Ying Tang., Wen-Bo Zheng. «Transurethral resection in women with symptomatic keratinizing squamous metaplasia of urinary bladder: a retrospective study of 92 cases» DOI: 10.1111/luts.12294. 2019.стр-137142