

Immunohistochemical Changes of Bcl-2 Protein in Bladder Leukoplakia

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Abstract In this work, indicators of the expression level of anti-apoptotic protein Bcl-2 in different stages of bladder leukoplakia were determined. The results showed that this protein was expressed only in the basal layer in the bladder lining epithelium in the control group. During the initial stage I of the development of leukoplakia, during 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 layer cells of the epithelium undergo metaplasia and are located vertically, Bcl-2 protein is relatively more expressed in the cells of the basal and intermediate layers, and in the III-stage of the disease, the expression of this protein increases even more.

Keywords Bladder, Cystitis, Leukoplakia, Immunohistochemistry, Bcl-2 protein

1. Introduction

The 6th protein of 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 inhibits the release of calcium ions and inhibits antioxidant activity by slowing down lipoperoxidation and NO-synthetase activity. The main function of Bcl-2 is to stop cytochrome S, AIF, ATF, which are anti-apoptotic molecules from mitochondria, from exiting through pores. Bcl-2 binds to the mitochondrial membrane and closes the 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 die 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 investigate the antiapoptosis 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 distribution and intensity of the immunohistochemical reaction in the sample were taken into account, i.e. (absence of expression or staining in a small amount of 10% of cells - 0 points, 10 to -25% - 1 point, 26 to -50% - 2 points, 51 to -75% - 3 points and More than 75% is -4 points.

2. Materials and Methods

The 6th protein of 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 inhibits the release of calcium ions and inhibits antioxidant activity by slowing down lipoperoxidation and NO-synthetase activity. The main function of Bcl-2 is to stop cytochrome S, AIF, ATF, which are anti-apoptotic molecules from mitochondria, from exiting through pores. Bcl-2 binds to the mitochondrial membrane and closes the pores, stops proapoptotic signals and prevents apoptosis [1,3,8].

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3. Results and 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 lining epithelium of the urinary bladder were studied in comparison with each other according to the clinical and morphological forms of leukoplakia and development periods.

In the control group, it was found that the lining epithelium of the bladder mucous membrane in the 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 oval and elongated. In the surface layers of the multi-layered epithelium, it was observed that the cells were relatively sparse, their nuclei were reduced in size, their staining was lighter, and their location was flattened. The results of the immunohistochemical examination for the detection of the anti-apoptosis protein of the epithelial cells showed that in the control group, this protein was expressed at a very low level in the cytoplasm of some cells located in the basement membrane and in the cambial level, and it was not expressed in the cells of the other intermediate and surface layers (Fig. 1).

The next task of the study was to study the expression level of anti-apoptotic protein Bcl-2 as the proliferative activity of epithelial cells increased in different periods of leukoplakia, and the following data were found. 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 it grew in bundles into the connective tissue layer under the basal layer epithelium.

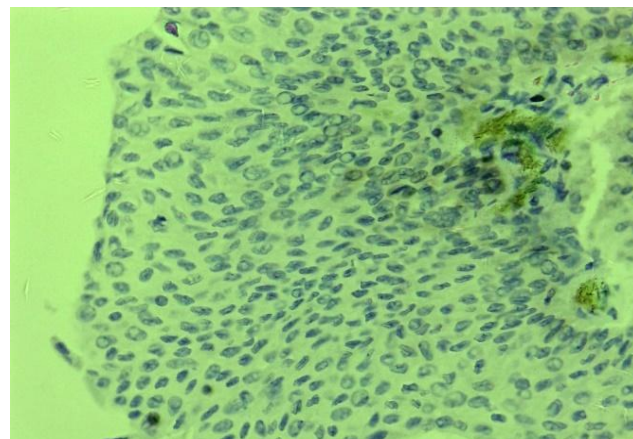


Figure 1. Bladder, norm, Bcl-2 protein is expressed at a low level in some cells of the basal layer. Stain: immunohistochemical method. 10x40

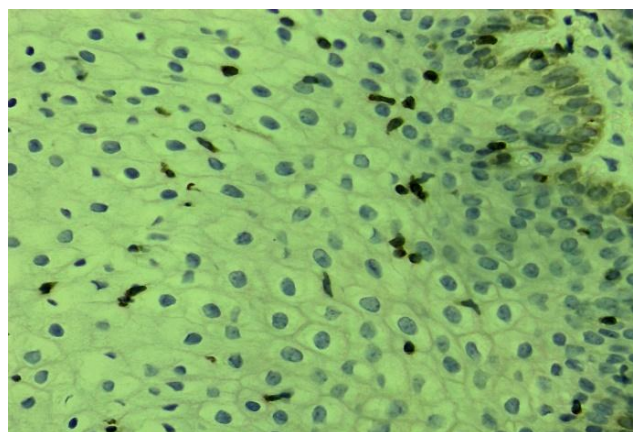


Figure 2. Bladder, leukoplakia grade I, Bcl-2 protein is expressed in some cells of the basal layer and intermediate layer. Stain: immunohistochemical method. 10x40

When the stratified variable epithelium was viewed as a whole, 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 a light brown cytoplasmic inclusion (Figure 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. When studied under a microscope lens, it was found that in the first period of leukoplakia, almost all the middle and superficial layers of the epithelium, except the epithelium of the basal layer, underwent metaplasia, that is, they were flattened, hydropic dystrophy, and vacuolated. As a result, Bcl-2 appeared in the nuclear membrane in the cytoplasm of some of the metaplastic cells, and a brown inclusion was found densely 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.

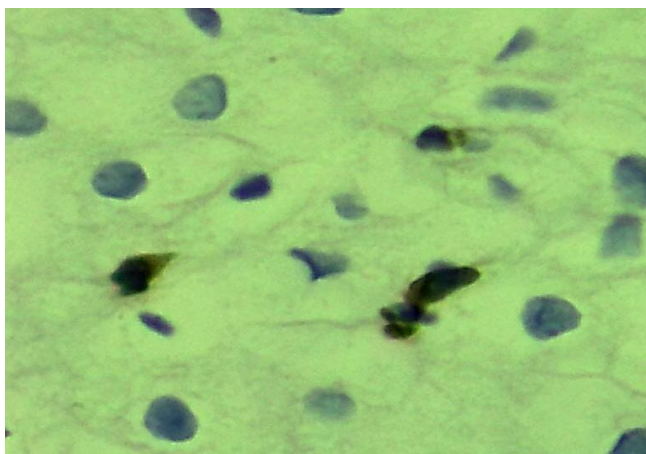


Figure 3. Bladder, leukoplakia grade I, Bcl-2 protein is expressed close to the nucleus of interstitial epithelial cells. Stain: immunohistochemical method. 10x40

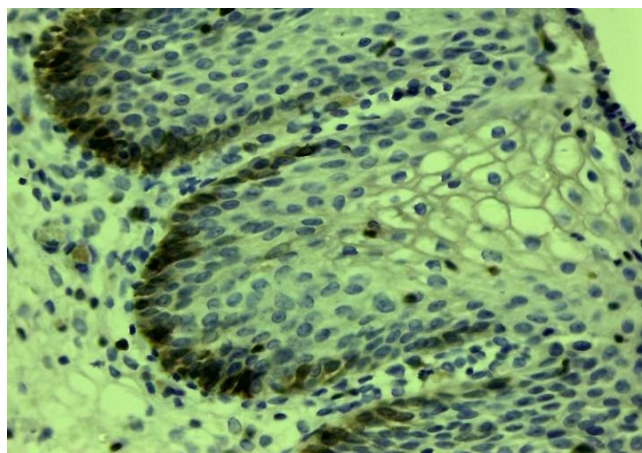


Figure 4. Urinary bladder, leukoplakia grade II, Bcl-2 is expressed in the basal layer 2-3 rows. Stain: immunohistochemical method. 10x40

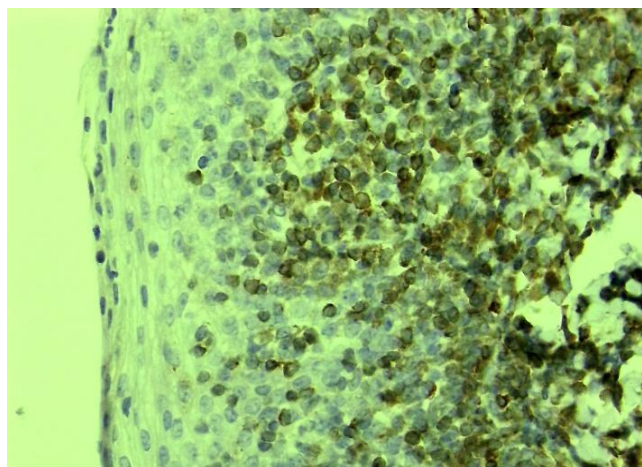


Figure 5. Bladder, leukoplakia grade III, Bcl-2 was expressed in most cells of the basal and intermediate layers. Stain: immunohistochemical method. 10x40

It was found that 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, 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, in this period Bcl-2 protein was expressed in a dark brown color in the cytoplasm of some of the cells of the 2-3 rows and intermediate layers located in the basal layer (Fig. 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 the cytoplasm was relatively narrow and light brown in color. Quantitatively, it was detected at the 3-point level, 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 from the previous stages is that proliferative activity and the process of metaplasia have developed in almost all the basal and intermediate rows of the epithelium. Another characteristic feature of this period is chronic inflammatory infiltrate in the private plate of sub-epithelial connective tissue, proliferation of blood vessel wall cells, and positive expression of Bcl-2 protein in them. In immunohistochemical examination, it is observed that Bcl-2 protein is expressed brown in cytoplasm and outer cytolemma, sticking to the nucleus of epithelial cells, this condition was evaluated with 3.5 points (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.

4. Conclusions

Immunohistochemical study of bladder leukoplakia, that is, determining which layers of the covering multi-layered variable epithelium express anti-apoptotic Bcl-2 protein, 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, indicating that the apoptosis activity is preserved in them.

During the first stage I of leukoplakia development, during the emergence of a metaplastic process in the changing epithelium, the expression of Bcl-2 protein in 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 stage 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.

In the III-period 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 increased from immunohistochemical markers in different stages of bladder leukoplakia, i.e. increased from 19.3% to 43.7% (3.5 points) as the level of the disease worsened. It is recommended to use these numbers as an indicator for predicting the consequences of the disease.

REFERENCES

- [1] Al-Shukri, S.H. General principles of treatment of bladder cancer patients. Importance of clinical, histological and biological prognostic factors for treatment selection / S.H. Al-Shukri, I.A. Korneev // *Practical Oncology*. 2004. - T. 4. - № 4. - PP. 204-211.
- [2] Zabolotneva AA, Gaifullin NM, Buzdin AA, Alekseev BA, Andreeva Yu, Shegai PV, Sokov DG, Rusakov IG Molecular markers of bladder cancer from the private to the whole. *Oncourology*. 2011. N 3. PP. 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*. - № 4. - PP. 30-34.
- [4] Matveev B.P. Clinical oncourology Verdana. - M.-2003. PP. 197-270.
- [5] Tumour markers in screening and monitoring of bladder cancer patients / S.P. Darenko, D.V. Perlin, V.N. Parshina, I.V. Chernyaev // *Oncourology*. - 2005. - № 3. - PP. 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). - PP. 169-175.
- [7] The role of bcl-2, p53 and Ki-67 index in predicting tumor recurrence for low grade superficial transitional cell bladder carcinoma / T.T. Wu, Y.H. Chen, Y.Y. Lee Y.R. Huang // *Y. Urol*. -200. - 163(3). - PP. 758-760.
- [8] McKenney JK. Precursor lesions of the urinary bladder. *Histopathology*. 2019; 74: PP. 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. pp -137142.