Central Asian Journal of Medicine

IMMUNOGYSTOCHEMICAL ASSESSMENT OF THE EXPRESSION OF KI-67 AND P53 MARKERS IN PEDIATRIC NEPHROPLASTOMA

Sarvar T. Islamov [1], Mirza-Ali A. Gafur-Akhunov [2], Khasan Z. Tursunov [3], Alisher B. Yigitaliyev [4]

1 Independent researcher, Department of "Hematology and Oncology" of the National Children's Medical Center, Tashkent, Uzbekistan.
E-mail: stimurovich@gmail.com

2 Doctor of Medical Sciences, Professor, Head of the Department of Oncological Diseases of the Center for the Development of Professional Qualifications of Medical Workers of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan
E-mail: gafurahunovmirzaali@gmail.com

3 Doctor of Medical Sciences, Professor, Head of the Department of Pathological Anatomy of the Tashkent Medical Academy, Tashkent, Uzbekistan
E-mail: tursunov.hasan@bk.ru

4 Assistant of the Department of Urology and Oncology of the Fergana Medical Institute of Public Health, Fergana, Uzbekistan
E-mail: ayigitaliyev6622@gmail.com

ABSTRACT

In this study, the levels of expression of immunohistochemical markers Ki-67 and p53 in various morphological variants of nephroblastoma (Wilms tumor) in children were studied. The study was conducted in 2020-2024 with the participation of 60 patients with nephroblastoma who were treated in specialized medical institutions of the city of Tashkent. Of these, 20 had epithelial, 20 had mesenchymal, and 20 had mixed forms of nephroblastoma. The Ki-67 marker was evaluated as a protein controlling the proliferative activity of tumor cells, and p53 as a protein controlling the processes of damage and apoptosis in the genome. According to the obtained results, a high degree of positive reactions prevailed in all variants. In particular, in the epithelial type, high expression was observed in 60% of cases for Ki-67 and in 40% of cases for p53. High proliferative and genetic activity was also noted in mesenchymal and mixed species. These indicators confirm the significance of immunohistochemical markers in assessing the aggressive course and prognosis of nephroblastoma.

Key words: Nephroblastoma, Wilms tumor, pediatric oncology, immunohistochemical analysis, Ki-67, p53, tumor morphology, tissue expression, proliferation, apoptosis.

INTRODUCTION

In pediatric oncology, nephroblastoma (Wilms' tumor) is one of the most common and malignant tumors. In general cases, it accounts for approximately 90-95% of all kidney tumors in children[3]. Its high biological activity, various morphological manifestations, and clinical course represent a major problem in the early diagnosis, treatment, and assessment of the prognosis of nephroblastoma.

The role of biomarkers regulating proliferative and apoptotic processes in nephroblastoma, in particular Ki-67 and p53 molecules, has become a relevant scientific direction in recent years. The Ki-67 proliferation index reflects active tumor growth and is used to assess the level of risk. Its high severity indicates rapid tumor growth and a high probability of recurrence [6,7]. At the same time, the p53 oncosuppressor protein participates in stopping the cell cycle, eliminating DNA mutations, and regulating apoptosis. Its mutation or high expression indicates the aggressiveness of the tumor, its resistance to treatment, and a poor prognosis [5,8].

Analysis of the level of expression of these biomarkers in various morphological variants of nephroblastoma (epithelial, mesenchymal, and mixed) contributes not only to a better understanding of the pathogenesis of the tumor, but also to the individualization of clinical treatment strategies. For example, for patients with a high Ki-67 index, the period of more aggressive chemotherapy or control can be shortened[2].

Also, leading oncology centers, in particular organizations such as SIOP (International Society of Paediatric Oncology) and COG (Children's Oncology Group), have proposed the inclusion of biomarkers such as Ki-67 and p53 in the standard pathomorphological assessment procedure [4,9]. Currently, based on these markers, "risk-stratified" treatment protocols for nephroblastoma are being developed. This provides an individual therapeutic approach for each patient with nephroblastoma.

In global and domestic studies, in particular in Russia, Europe, and the USA, cases with a high expression of Ki-67 >20% and p53 are indicated as one of the negative prognostic factors [1,2,6]. At the same time, studies combining molecular genetic and cytogenetic methods in conjunction with immunohistochemical analysis allow for a deeper understanding of tumor biology.

Therefore, determining the expression of Ki-67 and p53 markers in various histological variants of nephroblastoma has not only fundamental scientific significance, but also serves as an important clinical tool in practice for assessing the predisposition of patients to relapse, determining treatment tactics and control intervals. The widespread introduction of this into domestic clinical research is a pressing issue.

Materials and methods: To study pathomorphologically malignant tumors by the immunohistochemical method, patients with nephroblastoma (Wilms tumor) treated at the Tashkent City Branch of the Republican Specialized Scientific and Practical Medical Center of Surgery and Pediatric Medicine and the Tashkent City Branch of the Republican Specialized Scientific and Practical Medical Center of Surgery and Pediatric Medicine in 2020-2024 were examined. Of these, 20 patients with the epithelial form of nephroblastoma, 20 patients with the mesenchymal form, and 20 patients with the mixed form were selected for immunohistochemical examination. For an immunohistochemical study, the expression of Ki-67 and p53 markers was studied using an immunohistoprocessor from Bond Leca Australia (Australia).

Results:

The results of the study showed that in the epithelial type of Nephroblastoma, Ki-67-tumor cells was assessed as a percentage. The obtained nresults were evaluated as mild, moderate, and severe positive reactions. Of the 20 observed patients, 3 (15%) had a mild positive reaction to nephroblastoma, 5 (25%) had a moderate positive reaction, and 12 (60%) had a high positive reaction. No negative reaction processes were observed (Table 1).

Table 1.

Degree of proliferative activity of the Ki-67 reagent in the epithelial variant of nephroblastoma

No	Level	Number of patients (n=20)
1.	Low activity less than <10%	3 (15%)
2.	10-20% moderate activity	5 (25%)
3.	>20% high proliferative activity	12 (60%)

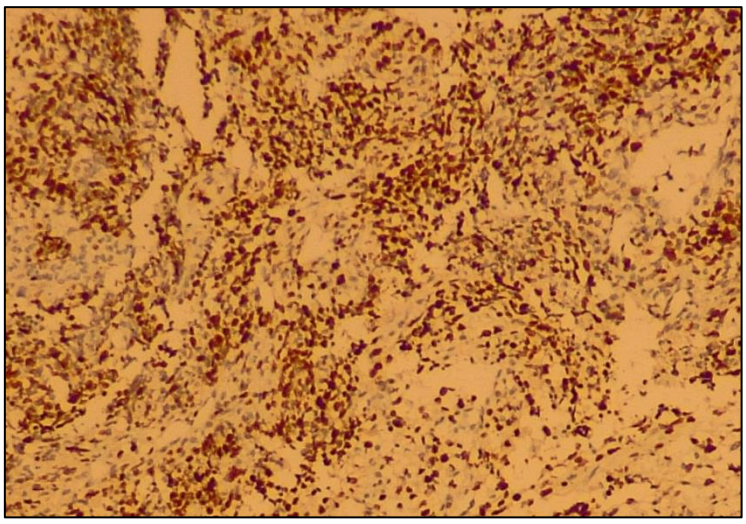

Fig. 1. In the epithelial type of nephroblastoma, there is a high degree of positive reaction to the reagent Ki67. IHCH - Dab chromogen. Ob10. Ok40.

In the epithelial type of nephroblastoma, dark brown staining of the tumor cell nuclei indicates the presence of the Ki-67 protein. Highly proliferative cells were detected in 12 (60%) of our studies. Moderate activity was 5 (25%) and mild activity was 3 (15%). It was established that in the epithelial type of nephroblastoma, the Ki-67 protein is abundant in the nuclei and the tumor has an aggressive course.

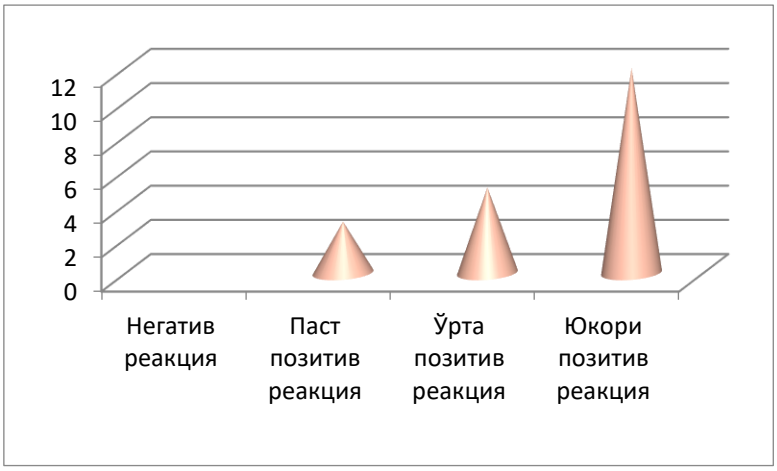

Fig. 2. Degree of proliferative activity of the Ki-67regent in the epithelial type of nephroblastoma (n=20).

20 patients with the epithelial type of nephroblastoma were selected, and the results obtained in all patients showed that the function of reagent p53 controls the presence of damage in the genome, which can lead to the further development of the pathology. The obtained results are evaluated in the form of mild, moderate, and severe positive reactions. Of the 20 selected patients, 4 (20%) had a mild positive reaction to nephroblastoma, 8 (40%) had a moderate positive reaction, and 8 (40%) had a high positive reaction (see Fig. 4.5).

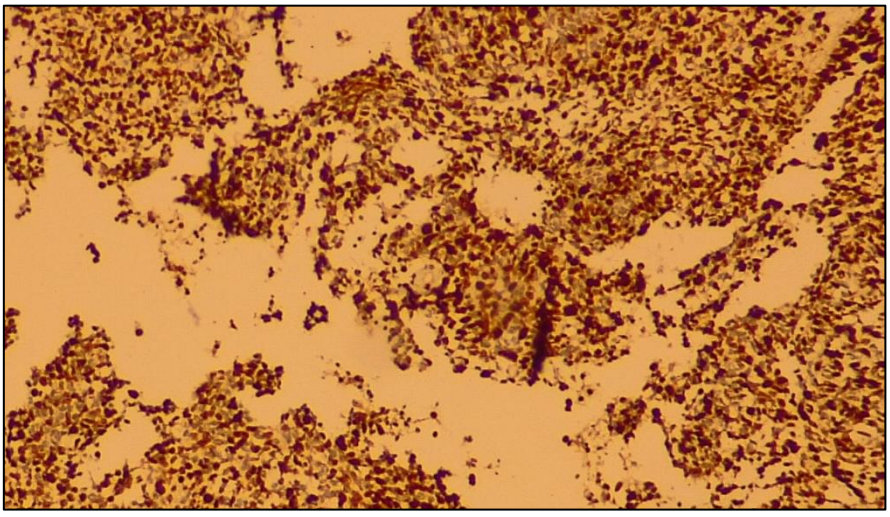

Fig. 3. In the epithelial type of nephroblastoma, a high degree of positive reaction to reagent p53 . IHCH - Dab chromogen. Ob10. Ok40.

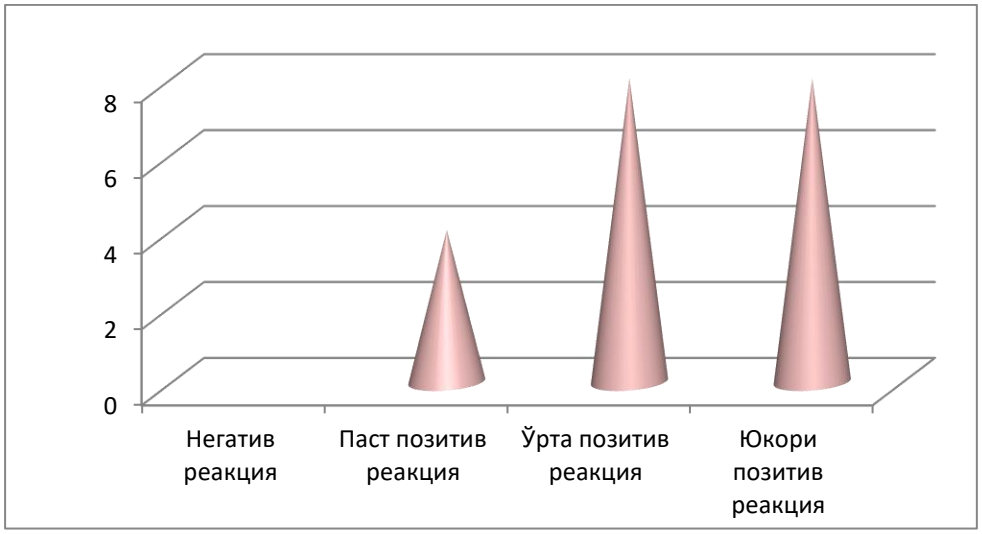

Fig. 4. Degree of proliferative activity of the p53 **reagent in the epithelial type of nephroblastoma (n=20).**

To determine the mesenchymal type of neuroblastoma, 20 patients with nephroblastoma were selected. The results obtained in all patients were evaluated as a percentage of the proliferative activity of Ki-67 tumor cells. Results were assessed as mild, moderate, and severe positive reactions. Of the 20 patients diagnosed with nephroblastoma, 3 (13%) had a mild positive reaction to nephroblastoma, 7 (35%) had a moderate positive reaction, and 10 (50%) had a high positive reaction.

Table 2.

Degree of proliferative activity of the Ki-67 reagent in the mesenchymal variant of nephroblastoma

No	Level	Patients **(n=20)**
1.	Low activity less than <10%	3 (15%)
2.	10-20% moderate activity	7 (35%)
3.	>20% high proliferative activity	10 (50%)

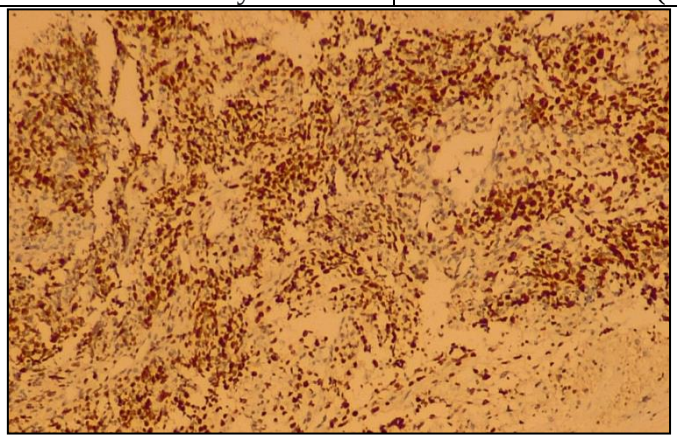

Fig. 5. In the mesenchymal type of nephroblastoma, a high positive reaction to the reagent Ki-67 is observed. IHCH - Dab chromogen. Ob10.Ok40.

Brown staining of the nucleus in the cells indicates a high level of proliferation. In our study, the mesenchymal level was elevated in 10 (50%) patients. The fact that the nuclei of the spindle cells are stained brown is clear evidence. (Fig. 6)

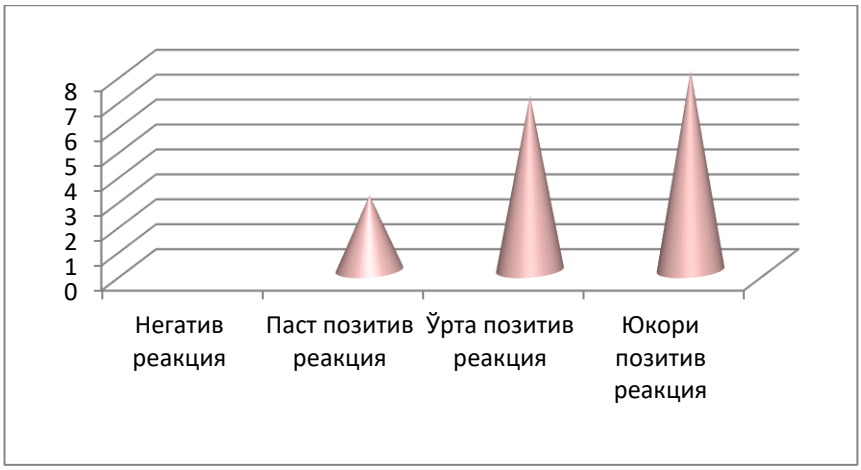

Fig. 6. Degree of proliferative activity of the Ki-67 reagent in the mesenchymal type of nephroblastoma .

In the mesenchymal type of nephroblastoma, the antigen for antibodies is the protein p53, which controls the course of cellular cycle processes, as well as the presence of damage in the genome, which can lead to the further development of the pathology. Of the 20 patients with the epithelial type of nephroblastoma, 2 (10%) had a mild positive reaction to nephroblastoma, 6 (30%) had a moderate positive reaction, and 12 (60%) had a high positive reaction.

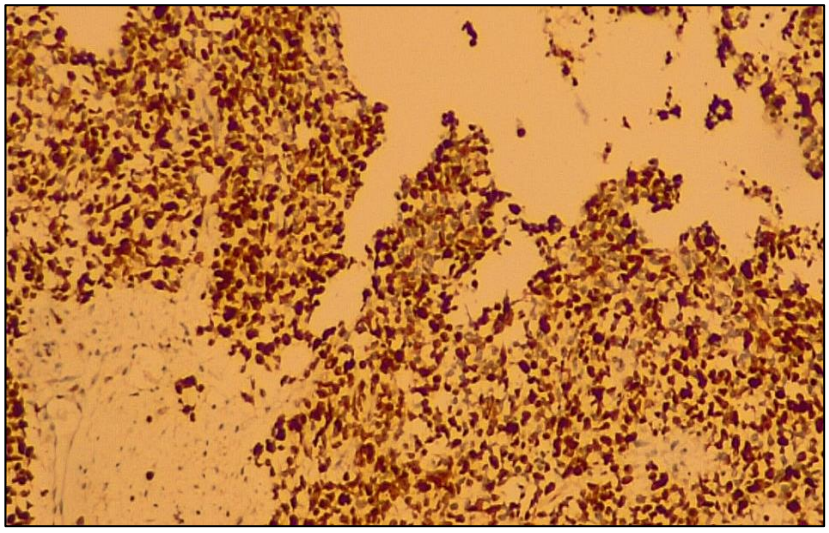

Fig. 7. In the mesenchymal type of nephroblastoma, a p53 **high degree of positive reaction to the reagent. IHCH - Dab chromogen. Ob10. Ok40.**

mixed type of nephroblastoma sign of proliferative activity of Ki-67 tumor cells n out of 20 patients diagnosed with nephroblastoma, a mild positive reaction was observed in 3 (15%) patients, a moderate positive reaction in 7 (35%) patients, and a high positive reaction in 10 (50%) patients (Table 3).

Table 3.

Mixed variant level of proliferative activity of the Ki-67 reagent in nephroblastoma

No	Level	Number of patients n=20
1.	Low activity less than <10%	%3
2.	10-20% moderate activity	7%
3.	>20% high proliferative activity	10 (%)

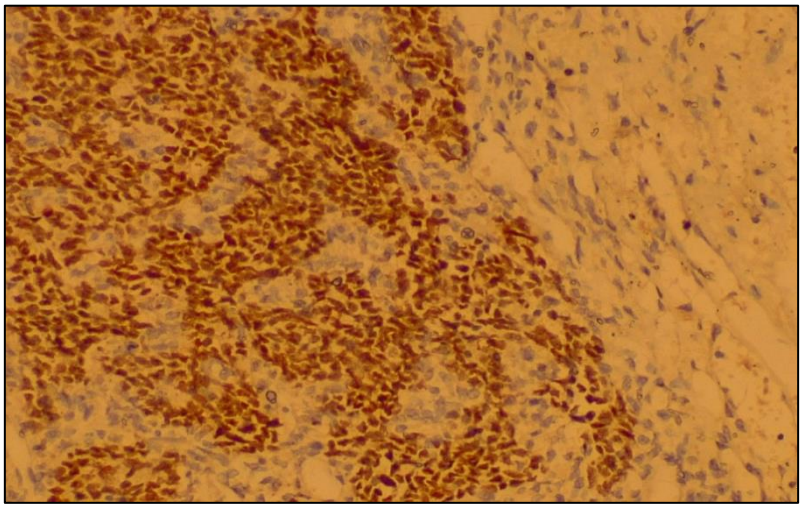

Fig. 8. In the mixed type of nephroblastoma, a high degree of positive reaction to the Ki-67 reagent . IHCH - Dab chromogen. Ob10. Ok40.

Microscopically, tumor cells with cellular polymorphism of epithelial origin and multiple foci of pathological mitosis are detected. Immunohistochemically, dark brown-stained malignant tumor cells are detected in the epithelial nuclei. In tumor nuclei, 70-80% of positively stained cell nuclei are visible.

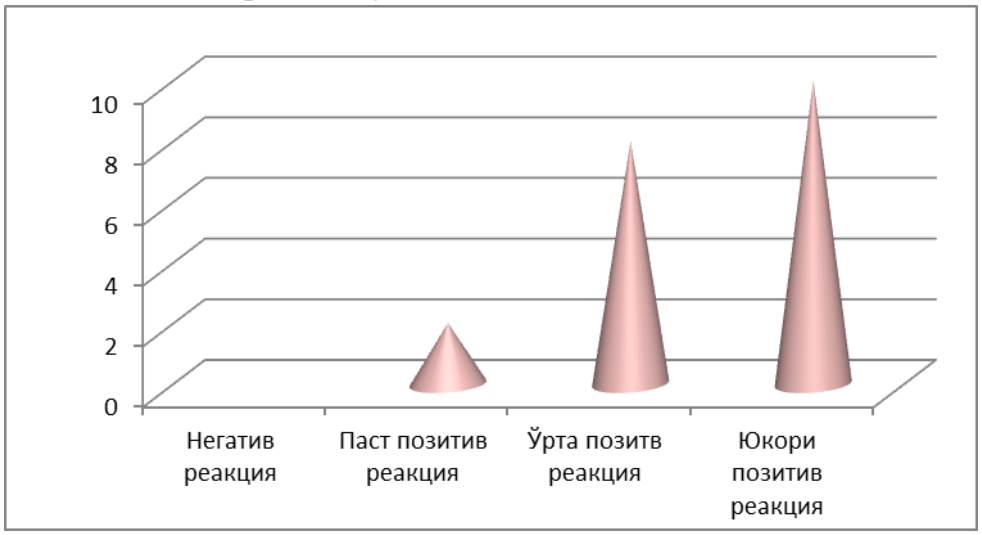

Fig. 9. Degree of proliferative activity of the Ki-67 reagent in the mixed type of nephroblastoma, n=20

In the mixed type - reagent p53, 20 patients with the mixed type of nephroblastoma were selected. The results obtained in all patients showed that of 20 patients, 4 (20%) had a mild positive reaction in nephroblastomas, 6 (30%) had a moderate positive reaction, and 12 (60%) had a high positive reaction **(**Fig. 10).

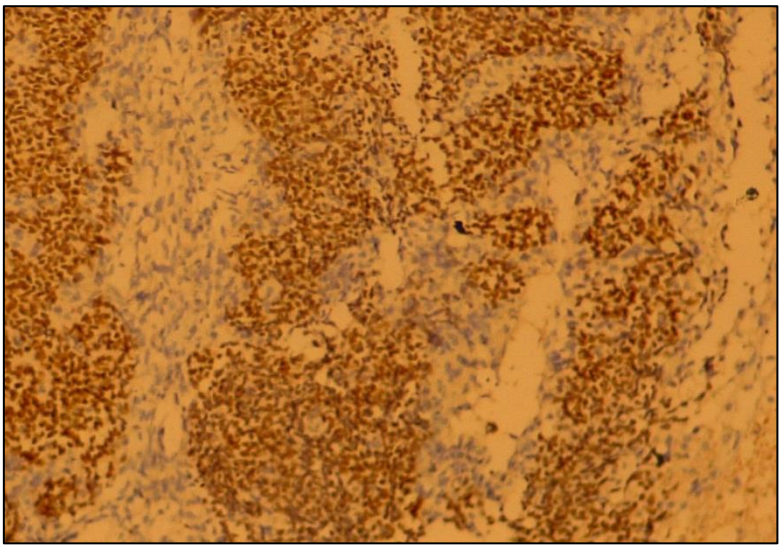

Fig.10. In the mixed type of nephroblastoma, a positive reaction of a high degree of p53 reagent . IHCH - Dab chromogen. Ob10. Ok40.

Conclusions:

In the epithelial type of nephroblastoma, the marker of proliferative activity of Ki-67-tumor cells was assessed as a percentage. The obtained nresults were evaluated as mild, moderate, and severe positive reactions. Of the 20 observed patients, 3 (15%) had a mild positive reaction to nephroblastoma, 5 (25%) had a moderate positive reaction, and 12 (60%) had a high positive reaction. In the epithelial type of nephroblastoma, dark brown staining of the tumor cell nuclei indicates the presence of the protein Ki-67. Highly proliferative cells were detected in 12 (60%) of our studies. Moderate activity was 5 (25%) and mild activity was 3 (15%). It was established that in the epithelial type of nephroblastoma, the Ki-67 protein is abundant in the nuclei and the tumor has an aggressive course.

20 patients with the epithelial type of nephroblastoma were selected, of which 4 (20%) had a mild positive reaction to nephroblastoma, 8 (40%) had a moderate positive reaction, and 8 (40%) had a high positive reaction.

The results obtained in all patients were evaluated as a percentage as a marker of the proliferative activity of Ki-67 tumor cells. Results were assessed as mild, moderate, and severe positive reactions. Of the 20 patients diagnosed with nephroblastoma, 3 (13%) had a mild positive reaction to nephroblastoma, 7 (35%) had a moderate positive reaction, and 10 (50%) had a high positive reaction. In the mesenchymal type of nephroblastoma, the antigen for antibodies is the protein p53, which controls the course of cellular cycle processes, as well as the presence of

damage in the genome, which can lead to the further development of the pathology. Of the 20 patients with the mesenchymal type of nephroblastoma, 2 (10%) had a mild positive reaction to nephroblastoma, 6 (30%) had a moderate positive reaction, and 12 (60%) had a high positive reaction.

Mixed type of nephroblastoma Signs of proliferative activity of Ki-67 tumor cells nOf the 20 patients diagnosed with euphroblastoma disease, 3 (15%) had a mild positive reaction to nephroblastoma, 7 (35%) had a moderate positive reaction, and 10 (50%) had a high positive reaction. In the mixed type - reagent p53, 20 patients with the mixed type of nephroblastoma were selected. The results obtained in all patients showed that of the 20 patients with nephroblastomas, a mild positive reaction was observed in 4 (20%) patients, a moderate positive reaction in 6 (30%), and a high positive reaction in 12 (60%) patients.

REFERENCES

1. Bahoush, G., Zareifar, S., Moradmand, S., & Movahedpour, A. (2020). Prognostic value of Ki-67 and p53 expression in Wilms tumor: a retrospective cohort study. BMC Cancer, 20(1), 1082.

2. Breslow, N. E., et al. (2018). Prognostic factors in Wilms tumor: results of the NWTS-5 study. Journal of Clinical Oncology, 36(15), 1510–1517.

3. Dome, J. S., Graf, N., Geller, J. I., Fernandez, C. V., Mullen, E. A., Spreafico, F., & Pritchard-Jones, K. (2015). Advances in Wilms Tumor Treatment and Biology: Progress Through International Collaboration. Journal of Clinical Oncology, 33(27), 2999–3007.

4. Gadd, S., Huff, V., Walz, A. L., et al. (2021). A Children's Oncology Group and TARGET initiative exploring the genetic landscape of Wilms tumor. Nature Genetics, 53(12), 1543–1553.

5. Khachatryan, S. G., Ivanov, V. A. (2021). Immunohistochemical expression of Ki-67 and p53 markers in kidney tumors in children<3. Russian Oncological Journal, 29(1), 47–52.

6. Parshina, T. A., Loginova, A. V., Kuzina, L. A. (2019). Ki-67 and p53 as prognostic markers in pediatric Wilms tumors. Pediatric Oncology Journal, 16(3), 205–210.

7. Rivera, M. N., Haber, D. A. (2005). Wilms' tumor: connecting tumor genesis and organ development in the kidney. Nature Reviews Cancer, 5(9), 699–712.

8. Stewart, C. J. R., Brennan, B. A., & Hammond, I. G. (2017). Evaluation of proliferation and apoptosis in pediatric kidney tumors using Ki-67 and p53 immunosuppressants. Histopathology, 60(2), 198–204.

9. Vujanić, G. M., Sandstedt, B., Harms, D., et al. (2002). Revised SIOP classification of kidney tumors of childhood. Medical and Pediatric Oncology, 38(2), 79–82.