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Results of immunogystochemic study in primary liver cancer developed on the basis of chronic viral hepatitis

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Abstract: This article is devoted to immunohistochemical studies associated with primary liver cancer (hepatocellular carcinoma - HCC) developed against the background of chronic viral hepatitis B, C. The topic is relevant, since HCC is one of the aggressive tumors with a high mortality rate, arising as a result of chronic hepatitis B and C, alcoholic or non-alcoholic fatty liver diseases. The study included 30 patients examined in 2020-2023, all of whom were diagnosed with viral hepatitis B or C. In the immunohistochemical study, molecular genetic markers (Ki-67, Bcl-2, VEGF, and p53) were studied. According to the results, in cases of HCC associated with viral hepatitis, high proliferative activity (Ki-67 >20%), apoptosis index (Bcl-2), angiogenesis activity (VEGF), and p53 gene suppressor mutations were observed more often. Molecular-genetic markers in primary liver cancer are important in assessing the aggressiveness of the tumor, the degree of metastasis, and the response to treatment. The research results open up new possibilities for improving the diagnosis, prognosis, and personalized therapy strategies for HCC.

Keywords: Hepatocellular carcinoma, Ki-67, Bcl-2, VEGF, p53 gene suppressor, immunohistochemical examination. Chronic hepatitis, liver cancer, proliferative activity, apoptosis, angiogenesis mutation.

Introduction: Relevance of the problem. Primary liver cancer (hepatocellular carcinoma, HCC) is one of the most aggressive and fatal malignant tumors. The development of this tumor is associated with chronic liver diseases, including: viral hepatitis B and C infections, alcoholic liver disease, non-alcoholic fatty liver disease [1,2].

In recent years, molecular genetic markers that allow

predicting the course of the disease, the likelihood of metastasis, and the response to therapy have been actively studied. The most important markers in primary liver cancer are Ki-67, Bcl-2, VEGF, and p53. These biomarkers play an important role in the mechanisms of: tumor cell proliferation, formation of new blood vessels (angiogenesis), cell death (apoptosis), which is very important for determining their diagnosis, prognosis, and treatment tactics [3,4,5].

Ki-67 - a marker of tumor proliferation activity - is a protein expressed in all phases of the cell cycle (except for the G0 phase) and is an important marker of the degree of cell proliferation. High Ki-67 expression is associated with tumor aggressiveness, rapid growth, and a poor prognosis [6]. Studies show that high Ki-67 expression in HCC is associated with: high mitotic activity, rapid progression (development), low-grade differentiated tumors, shortened patient lifespan [7.8], high Ki-67 levels can be an independent prognostic marker in the late stages of the disease [9]. Patients are classified based on Ki-67 expression: patients with high Ki-67 levels require aggressive treatment methods, including targeted therapy and systemic treatment [10].

Bcl-2 - a protein that regulates apoptosis (B-cell lymphoma 2) - is an anti-apoptotic protein that prevents apoptosis (natural cell death). Disruption of apoptosis leads to the survival of cancer cells [11]. Studies show that Bcl-2 expression was detected in 40-60% of HCC cases. Its high level is associated with: resistance of cancer cells to apoptosis, low sensitivity to chemotherapy, and a high risk of relapse [12,13]. Determination of the Bcl-2 level allows predicting the sensitivity of the tumor to treatment. Bcl-2 inhibitors (for example, venetoclax) are considered promising drugs in the treatment of HCC [14].

VEGF - angiogenesis factor (vascular endothelial growth factor) - the main protein regulating angiogenesis, stimulating the formation of new blood vessels for the blood supply of the tumor [15]. High VEGF expression is associated with: active angiogenesis, high vascularization (blood supply) of the tumor, a high risk of metastasis, and a shorter patient lifespan [16,17]. Anti-VEGF therapy (bevatsizumab, ramutsirumab) is used in the treatment of HCC and improves the prognosis in patients with high levels of VEGF [18].

p53 - tumor-suspending protein - is a protective protein against tumors that controls the cell cycle and regulates apoptosis. p53 mutations lead to uncontrolled cell proliferation and cancer development [19]. p53 mutations have been detected in 25-50% of HCC cases. impaired p53 function is associated with tumor aggressiveness, therapy resistance, and the risk of metastasis [20]. determining p53 levels helps predict treatment response. MDM2 inhibitors (negative regulators of p53) are considered as promising targeted therapy for HCC [21].

Thus, molecular genetic markers Ki-67, Bcl-2, VEGF, and p53 play an important role in the pathogenesis and development of hepatocellular carcinoma (HC). Their study will allow improving the diagnosis of the disease and predicting its course, choosing personalized therapy, and improving the development of new targeted drugs for HCC [18,19,20]. Further study of these biomarkers is necessary for the early detection of primary liver cancer and the development of effective treatment strategies.

METHODS

In the study, 30 patients with primary liver cancer examined and treated at the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology and its Tashkent regional branch underwent retrospective and prospective immunohistochemical examination in 2020-2023. In this case, all patients had a history of viral hepatitis B and C and received treatment. Later, after chronic hepatitis, he was diagnosed with primary hepatocellular carcinoma and hospitalized. In these patients, the results of histological examination and immunohistochemical studies were studied. Pathomorphologically, the use of the immunohistochemical method in hepatocellular liver cancer, in which the study of molecular genetic markers is carried out for the first time, reveals its deeper morphologically significant features in hepatocellular liver cancer developed against the background of chronic viral hepatitis B,C, plays a special role in determining treatment tactics and predicting the disease.

Biopsies (trepan-biopsy, surgical material) obtained from all patients were taken from paraffin blocks, processed for immunohistochemical examination, and examined by taking sections on a slide. The Ki67, bcl 2, VHFR, and w p53 genes were studied by immunohistochemical examination. The conducted immunohistochemical examination method was technically carried out as follows (Table No1).

Table No. 1

Stages of immunohistochemical (IHC) examination.

№	Procedure	Reagents	Duration

1	Prepare sections 4 µm thick	Polylysine-coated slides	
2	Drying sections		Room temperature 24 hours
3	Drying in a thermostat		T: 55-60°C, 60 minutes
4	Deparaffinization	Ortho-xylene	10 minutes x 3 times
5	Dehydration	96% ethanol	3 minutes x 3 times
6	Rehydration	Distilled water	10 minutes
7	Antigen retrieval (Demasking)	Demasking buffer, T: 98°C	30-40 minutes
8	Washing	Tris-buffer solution (pH 7.5)	5 minutes
9	Blocking endogenous peroxidase activity	3% hydrogen peroxide	5 minutes
10	Washing	Distilled water	3 minutes
11	Incubate with primary antibodies	Specific antibodies	20-30 minutes
12	Washing	Tris-buffer solution (pH 7.5)	5 minutes
13	Incubate with secondary antibodies (detection)	Visualization system	20-30 minutes
14	Washing	Tris-buffer solution (pH 7.5)	5 minutes
15	Visualization with DAB	DAB-chromogen	5 minutes
16	Washing	Distilled water	3 minutes
17	Counterstaining	Mayer's hematoxylin	5 minutes
18	Washing	Tap water	1 minute
19	Dehydration	96% ethanol	2 times x 5 minutes
20	Clearing (Despiriting)	Ortho-xylene	2 times x 5 minutes
21	Mounting	Balsam, cover slip	

For immunohistochemical examination, the expression of Ki67, Vcl2, VGFR and w p53 monoclonal antibodies in cells was studied using an immunohistoprocessor "Bond Leica Australia" (Australia).

In our study, the study of the expression of molecular structures in liver cancer cells led to the development of viral hepatitis B, C in hepatocellular carcinoma.

RESULTS

The Ki67 indicator morphologically characterized the staining of the cell nucleus in

hepatocellular liver cancer as follows: the obtained results were assessed as a mild, moderate, and severe positive reaction. The results of the study showed that out of 30 patients, 5 (16.6%) had a mild positive reaction, 10 (33%) had a moderate positive reaction, and 15 (50%) had a high positive reaction. No negative reaction processes were observed (Table No2).

Table No 2

Ki67 indicators in hepatocellular carcinoma associated with viral hepatitis B, C

№	Level	Patients (N=30)
1	<10% low activity	5 (16.6%)
2	10-20% moderate activity	10 (33%)
3	>20% high proliferative activity	15 (50%)

Microscopically, liver cells with hyperplasia and polymorphism of hepatocytes, trabeculae are not preserved, malignant tumor cells form tumor islands of polymorphic hepatocytes with pathological mitosis and cell necrobiosis. The nuclei of tumor cells are stained dark brown (Fig. 1).

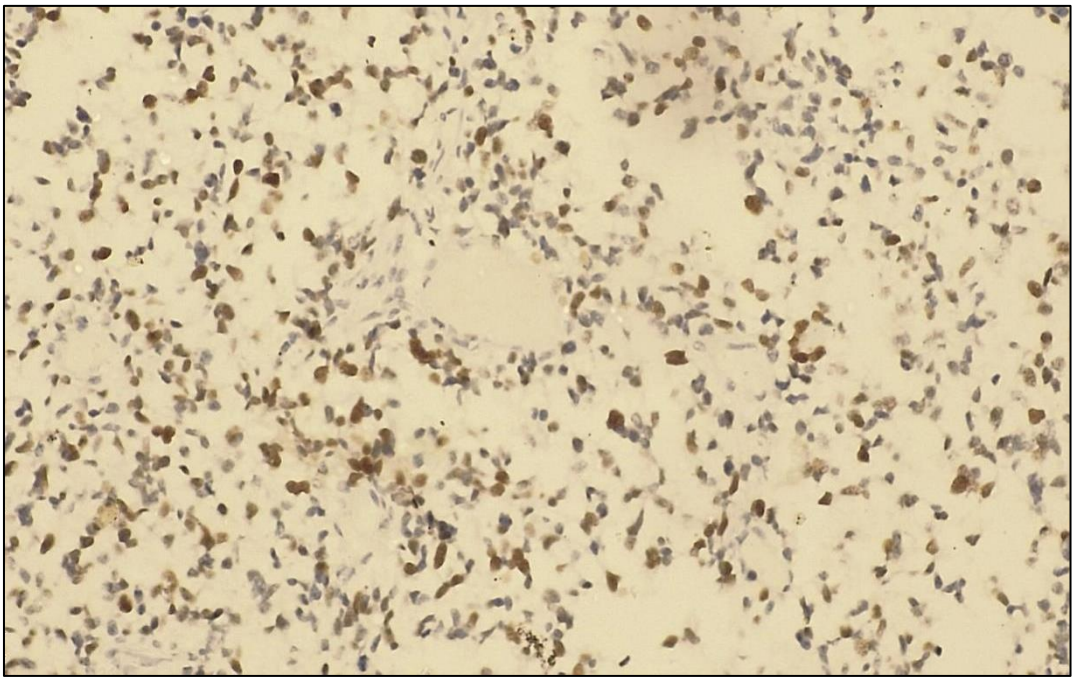

Figure 1. A high degree of positive response to the Ki 67 marker in patients diagnosed with hepatocellular carcinoma, viral hepatitis B (80%). IHC - Dab chromogen. Ob10. Ok40.

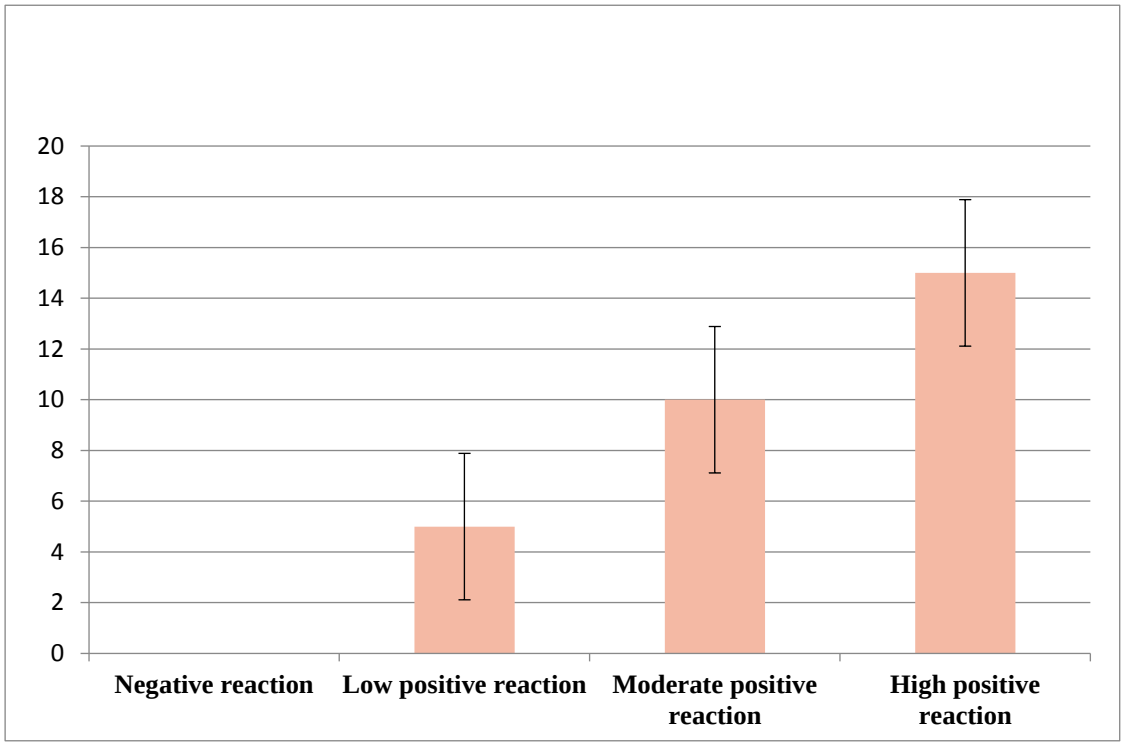

Figure 2. Degree of proliferative activity of tumor cells when hepatocellular carcinoma develops against the background of viral hepatitis B.

To determine tumor apoptosis in hepatocellular carcinoma with viral hepatitis B, the Vcl2 indicator was studied in 30 patients. In patients, the Vcl2 marker was used to determine tumor apoptosis, which regulates cell death by controlling the permeability of the mitochondrial membrane. The obtained results were evaluated using the ALLRED method. It looks at how many percent of the system's cells are positive for their receptors and how well the receptors appear after staining. These data are then combined to evaluate the sample on a scale from 1 to 3. In this case, the minimum score is 0 (negative), 1 point (low positive 10-30%), 2 points (medium positive 30-60%), 3 points (high positive 60-100%). Of the 30 selected patients, 4 (13.3%) had a mild positive reaction, 12 (40%) had a moderate positive reaction, and 14 (46%) had a high positive reaction (Table 3).

Table No. 3

Positive reaction of the Bcl-2 marker in patients with viral hepatitis B,C in hepatocellular carcinoma

№	Level	Patients (N=30)
1	<10% low activity	4 (13.3 %)
2	10-20% moderate activity	12 (40 %)
3	>20% high proliferative activity	14(46 %)

Microscopically: liver cells with hyperplasia and polymorphism of hepatocytes, trabeculae are not preserved, malignant tumor cells form tumor islands of polymorphic hepatocytes with pathological mitosis and cell necrobiosis. The cell membranes of malignant tumors are stained dark brown.

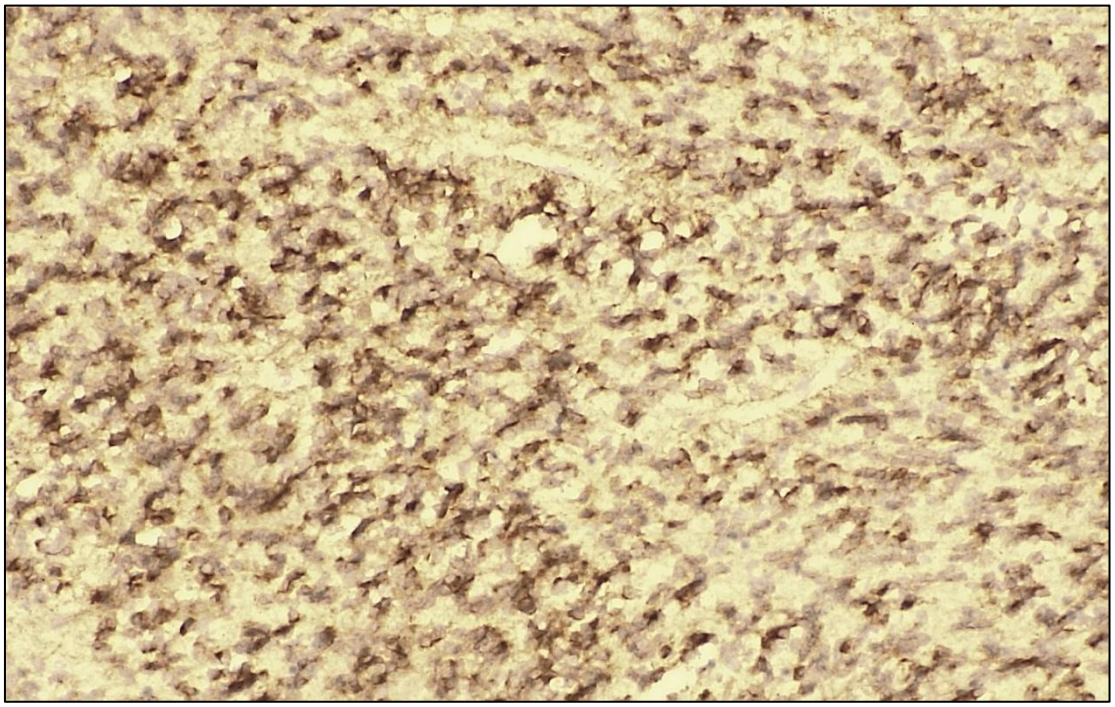

Figure 3. High positive reaction of the Bcl-2 marker in patients diagnosed with viral hepatitis B in hepatocellular carcinoma. (70%). IHC - Dab chromogen. Ob10. Ok40.

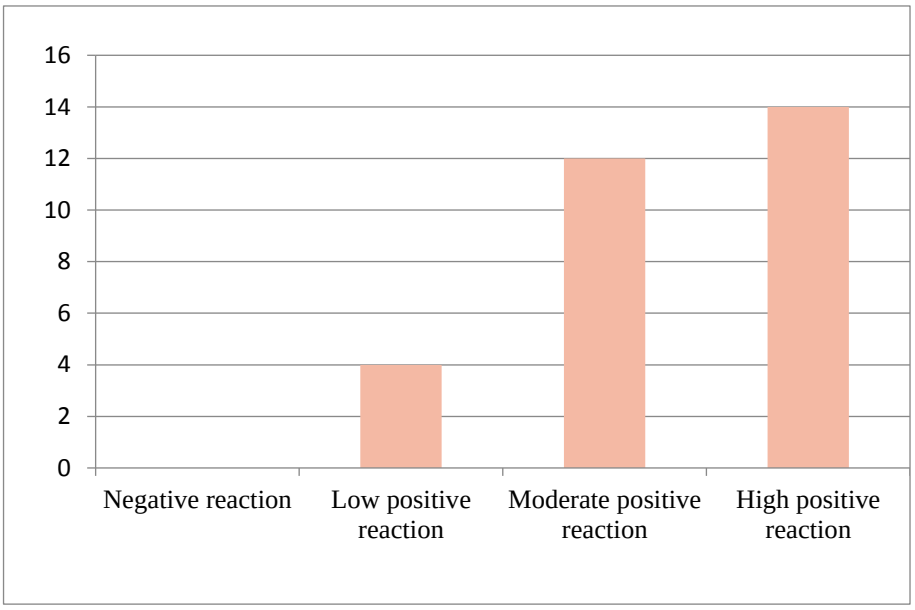

Figure 4. Level of Bcl-2 marker expression in patients diagnosed with viral hepatitis B in hepatocellular carcinoma.

The results of w p53 expression in hepatocellular carcinoma with viral hepatitis B were assessed using the ALLRED method. It considers what percentage of system cells are positive for receptors and how well receptors appear after staining. Then these data were combined for a sample assessment on a scale from 1 to 3. The minimum score was 0 (negative), 1 point (low positive 10-30%), 2 points (medium positive 30-60%), 3 points (high positive 60-100%). Of the 30 selected patients, 7 (23.3%) had a mild positive reaction, 10 (13.3%) had a moderate positive reaction, and 13 (43.3%) had a high positive reaction (Table 4).

Table No4.
Level of expression of the p53 marker in hepatocellular carcinoma against the background of viral hepatitis C.

№	Level	Patients (N=30)
1	1 point low position reaction (10-30%)	7 (23.3 %)
2	2 points average positive reaction (30-60%)	10 (33.3 %)
3	3 points high positive reaction (60-100%)	13 (43.3 %)

When viewed under a microscope, liver cells with hyperplasia and polymorphism of hepatocytes, trabeculae are not preserved, malignant tumor cells form tumor islands of polymorphic hepatocytes with pathological mitosis and cell necrobiosis. The nuclei of malignant tumor cells are stained dark brown (Fig. 3).

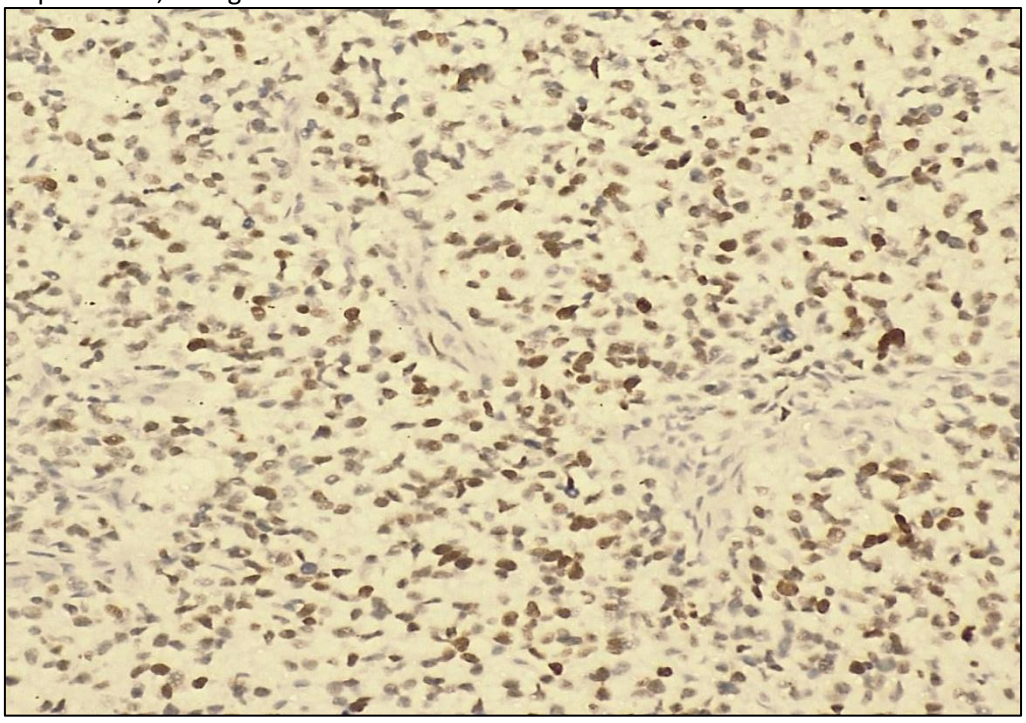

Figure 5. High positive reaction of the p53 marker in patients diagnosed with viral hepatitis B in hepatocellular carcinoma. (90%). IHC - Dab chromogen. Ob10. Ok40.

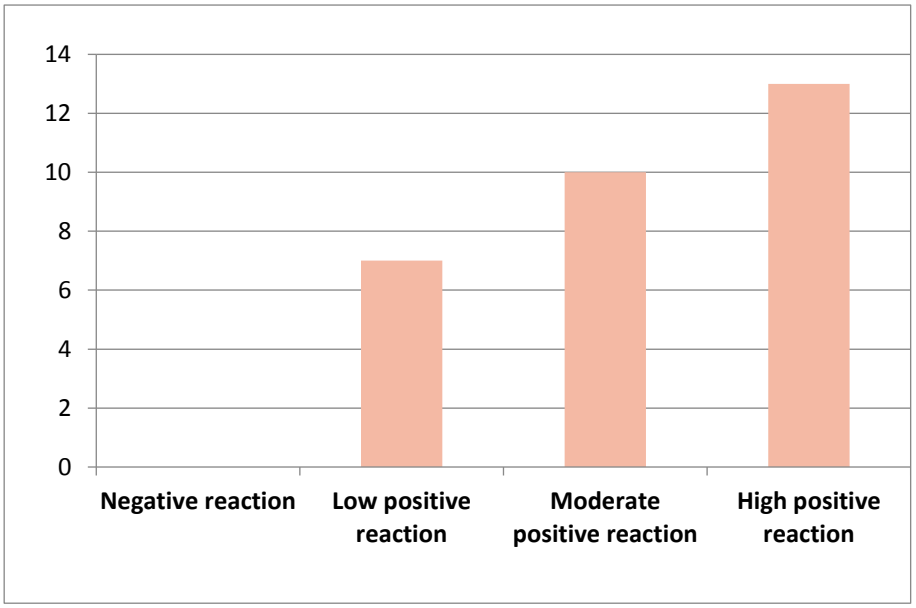

Figure 6. Level of the p53 marker in patients diagnosed with viral hepatitis C in hepatocellular carcinoma.

In detected hepatocellular carcinoma of viral hepatitis C. The obtained results were considered in what percentage of cells the markers were positive and how expressive they were after staining. These data were then combined to evaluate the sample on a scale from 1 to 3. The minimum score is 0 (negative), 1 point (low positive 10-30%), 2 points (medium positive 30-60%), 3 points (high positive 60-100%). Of the 30 selected patients, 8 (26.6%) had a mild positive reaction, 12 (40%) had a moderate positive reaction, and 10 (33.3%) had a high positive reaction (Table 5).

Table 5.

Level of expression of the VGFR marker in patients with viral hepatitis C in hepatocellular carcinoma

№	Level	Patients (N=30)
1	1 point low position reaction (10-30%)	8 (26.6 %)
2	2 points average positive reaction (30-60%)	12 (40 %)
3	3 points high positive reaction (60-100%)	10 (33.3 %)

with

Microscopically: liver cells with hyperplasia and polymorphism of hepatocytes, trabeculae not preserved, malignant tumor cells form tumor islands pathological mitosis of polymorphic hepatocytes and cell necrobiosis. The endothelium of blood vessels is stained dark brown.

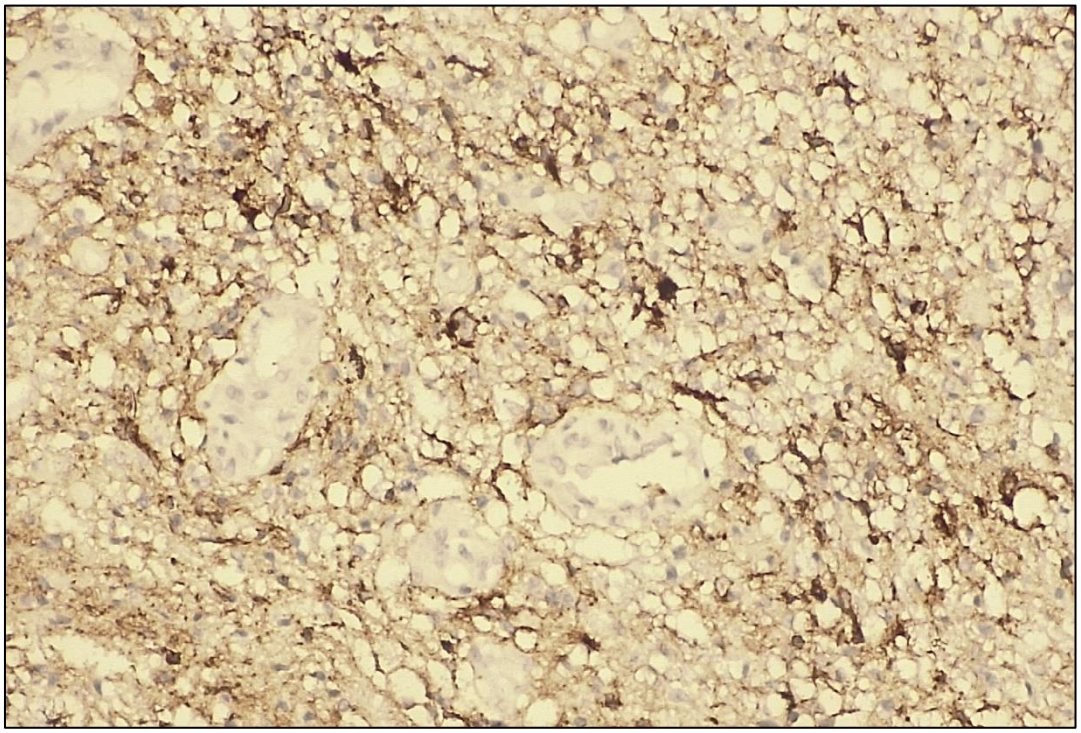

Figure 7. High positive reaction of the VHFR marker in patients diagnosed with viral hepatitis B in the light cell type of hepatocellular carcinoma. (90%). IHC - Dab chromogen. Ob10. Ok40.

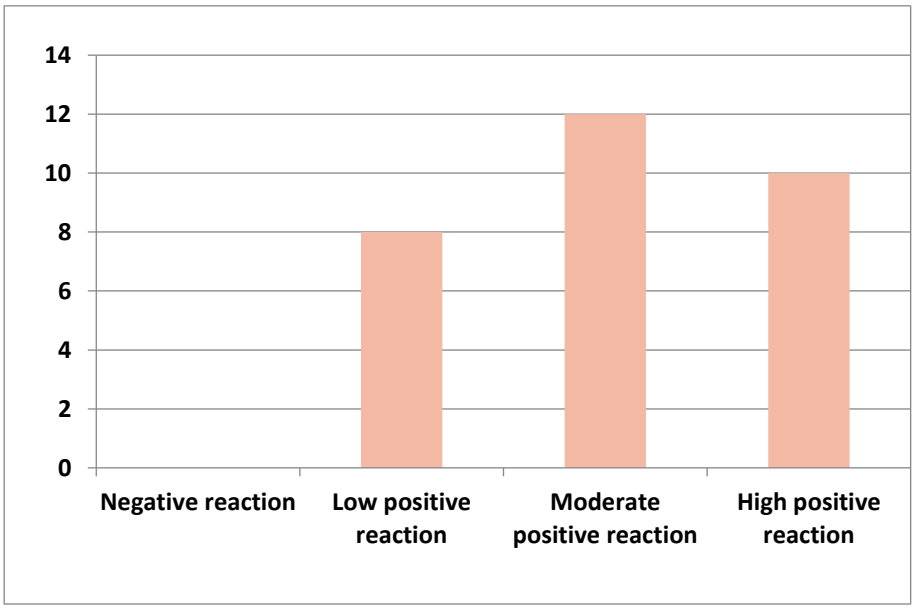

Figure 8. The level of the VHFR marker in patients with viral hepatitis C in hepatocellular carcinoma.

CONCLUSION

In our study, 30 patients were selected for immunohistochemical examination of patients with viral hepatitis B, C diagnosed with hepatocellular carcinoma. The results obtained in all patients were evaluated as a percentage of the marker of proliferative activity of Ki 67 tumor cells.

The obtained results were assessed as mild, moderate, and severe positive reactions. Of the 30 observed patients, 5 (16.6%) had a mild positive reaction, 10 (33%) had a moderate positive reaction, and 15 (50%) had a high positive reaction. No negative reaction processes were observed.

For the purpose of detecting tumor apoptosis Bcl-2 in hepatocellular carcinoma with identified viral hepatitis B, C. Of the 30 selected patients, 4 (13.3%) had a mild positive reaction, 12 (40%) had a moderate positive reaction, and 14 (46%) had a high positive reaction.

In hepatocellular carcinoma with viral hepatitis B, out of 30 patients selected for the gene suppressor - p53, 7 (23.3%) had a mild positive reaction, 10 (33.3%) had a moderate positive reaction, and 13 (43.3%) had a high positive reaction.

VHFR, a signaling protein produced by cells to stimulate angiogenesis in hepatocellular carcinoma with viral hepatitis C, was studied.

Of the 30 selected patients, 8 (26.6%) had a mild positive reaction, 12 (40%) had a moderate positive reaction, and 10 (33.3%) had a high positive reaction.

The results of the study showed that in hepatocellular carcinoma developed against the background of viral hepatitis B, C, the indicators of molecular genetic markers Ki 67, Bcl-2 gene-suppressor p53 and VEGF are high, which are considered factors negatively affecting the tactics and prognosis of its progression.

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