FEATURES OF AFP EXPRESSION LEVEL AT DIFFERENT STAGES AND GRADATIONS OF HCC, CORRELATION ANALYSIS BETWEEN SERUM AFP

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Abstract

Hepatocellular carcinoma (HCC) is a malignant tumor of the liver which accounts for up to 90% of all liver cancers.

In recent years, there has been an increase in the incidence of HCC all over the world, including in Kazakhstan. Diagnostic issues are still important. Alpha-fetoprotein (AFP) is a specific marker most widely used in the diagnosis of HCC. The article describes of the features of the AFP expression level in immunohistochemical studies with different stages and gradation of hepatocellular carcinoma, as well as a correlation analysis with serum AFP.

***Material and methods.** A total of 50 patients with HCC were analyzed. Blood serum tests were performed to determine the level of AFP and an IHC study to assess the expression of AFP.*

***Results.** When analyzing the serological AFP, it was found that in the vast majority of cases (n=33), values were between 10-20 units/ml. In 83% cases HCC, cytoplasmic and nuclear expression of AFP was determined in malignant cells in IHC. The expression of the AFP was high in 32% cases, moderate in 46% cases, and low or not detected in 22% cases. The area of AFP - immunopositive cells node averaged 37.25±15.47%. When conducting a correlation analysis, it was found that the overall Pearson correlation coefficient between serum AFP and the degree of AFP staining was r = +0.0089.*

***Conclusion.** Critically high AFP values correlate with the degree of HCC differentiation. The results of IHC showed that in 83% of patients with HCC, cytoplasmic and nuclear expression of AFP, which indicates a high sensitivity of the marker regarding the definition of malignancy. Given the absence of a correlation, it can be assumed that the serum AFP value cannot be associated with AFP expression data in immunohistochemistry and can be used as a separate value for HCC differentiation.*

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The authors declare that they have no conflicts of interest

Keywords
hepatocellular carcinoma, serum alpha-fetoprotein, immunohistochemistry, stage, grade

Гепатоцеллюлярлық карциноманың түрлі сатылары мен градацияларындағы афп экспрессиясы деңгейінің ерекшеліктері, сарысулық афп арасындағы корреляциялық талдауы

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Аңдатпа

Гепатоцеллюлярлы карцинома - гепатоциттерден пайда болатын бауырдың қатерлі ісігі, бауырдың барлық қатерлі ісіктерінің 90% құрайды.

Соңғы жылдары бүкіл әлемде, оның ішінде Қазақстандада гепатоцеллюлярлық карциноманың алғашқы анықталған жағдайларының кездесу жиілігінің артуы байқалады. Қазіргі уақытта диагностикалық мәселелер әлі де маңызды болып қала береді. Альфафетопротеин ерекше маркер, ГЦК диагнозында кеңінен қолданылады. Мақалада әртүрлі сатымен және гепатоцеллюлярлық карциноманың градациясымен иммуногистохимиялық зерттеу кезінде АФП экспрессия деңгейінің ерекшеліктерін талдау, сондай-ақ сарысулық АФП-мен корреляциялық талдау сипатталады.

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Мүдделер қақтығысы
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Материал және әдістер. *Ашық хирургиялық ота жоспарлаған ГЦК-ы бар 50 пациенттің деректері талданды. Барлық пациенттерде АФП деңгейін анықтау үшін қан сарысуын зерттеу және АФП экспрессиясын бағалау үшін ИГХ зерттеу жүргізілді.*

Нәтижелер. *Серологиялық АФП деңгейін талдау кезінде басым көпшілік жағдайларда (n=33) АФП мәндері 10-20 бірлік/мл арасында болғаны анықталды, жүргізілген иммуногистохимиялық зерттеулердің нәтижелері ГЦК-ы бар науқастардың 83% - ында қатерлі жасушаларда АФП цитоплазмалық және ядролық экспрессиясы анықталатынын көрсетті. ГЦК түйініндегі АФП маркерінің экспрессия деңгейі 32% (n=16) жағдайда жоғары, 46% (n=23) жағдайда - орташа және 22% (n=11) жағдайда - төмен немесе мүлдем анықталмаған. ГЦК торабындағы АФП-иммунопозитивті жасушалардың ауданы орта есеппен 37,25±15,47% - ды құрады. Корреляциялық талдау жүргізу кезінде сарысулық АФП мен ИГХ-дағы АФП бояу дәрежесі арасындағы Пирсонның жалпы корреляция коэффициенті r = +0,0089 құрағаны анықталды.*

Қорытынды. *АФП-нің сыни жоғары мәндері ГЦК саралау деңгейімен байланысты. ИГХ нәтижелері патологиялық жасушаларда ГЦК бар науқастардың 83%-ында α-фетопротеиннің цитоплазмалық және ядролық экспрессиясы анықталатынын көрсетті, бұл қатерлі ісікті анықтауға қатысты маркердің жоғары сезімталдығын көрсетеді. Корреляциялық байланыстың жоқтығын ескере отырып, сарысулық АФП мәні иммуногистохимия кезінде АФП экспрессиясының деректерімен байланысты болмайды және ГЦК дифференциалдау үшін жеке мән ретінде қолданылуы мүмкін деп болжауға болады.*

Түйін сөздер
гепатоцеллюлярлық карцинома, сарысулық альфафетопротеин, иммуногистохимия, сатысы, градациясы

Особенности уровня экспрессии афп при различных стадиях и градациях гцк, корреляционный анализ между сывороточным афп

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Аннотация

Гепатоцеллюлярная карцинома – злокачественная опухоль печени, происходящая из гепатоцитов, составляет до 90% всех раков печени. В последние годы наблюдается увеличение частоты встречаемости первично выявленных случаев гепатоцеллюлярной карциномаы во всем мире, в том числе и в Казахстане. В настоящее время вопросы диагностики остаются все еще важными. Альфафетопротеин специфичный маркер, наиболее широко используемый в диагностике ГЦК. В статье описывается анализ особенностей уровня экспрессии АФП при иммуногистохимическом исследовании с различной стадией и градацией гепатоцеллюлярной карциномы, а также корреляционный анализ с сывороточным АФП.

Материал и методы. *Всего были проанализированы данные 50 пациентов с ГЦК, которым планировались открытые хирургические вмешательства. У всех пациентов были выполнены исследования сыворотки крови для определения уровня АФП и ИГХ исследование для оценки экспрессии АФП.*

Результаты. *При анализе уровня серологического АФП было выявлено, что в преобладающе большинстве случаев (n=33) значения АФП находились между 10-20 ед/мл. Результаты проведенных иммуногистохимических исследовании показали, что у 83% больных с ГЦК в злокачественных клетках определяется цитоплазматическая и ядерная экспрессия АФП. Уровень экспрессии маркера АФП в узле ГЦК в 32% (n=16) случаях был высоким, в 46% (n=23) случаях - умеренным, и в 22% (n=11) случаях - низким или же вовсе не определялся. Площадь АФП - иммунопозитивных клеток в узле ГЦК в среднем составила 37,25±15,47%. При проведении корреляционного анализа было выявлено, что общий коэффициент корреляции Пирсона между сывороточной АФП и степенью окрашивания АФП на ИГХ составил r = +0,0089.*

Заключение. *Критически высокие значения АФП коррелируют со степенью дифференцировки ГЦК. Результаты ИГХ показали, что у 83% больных с ГЦК в патологических клетках определяется цитоплазматическая и ядерная экспрессия α-фетопротеина, что свидетельствует о высокой чувствительности маркера касательно определения злокачественности. Учитывая отсутствия корреляционной связи, можно предположить, что значение сывороточной АФП не могут быть ассоциированы с данными экспрессии АФП при иммуногистохимии и могут быть применены как отдельное значение для дифференцировки ГЦК.*

Конфликт интересов
Авторы заявляют об отсутствии конфликта интересов

Ключевые слова
гепатоцеллюлярная карцинома, сывороточный альфафетопротеин, иммуногистохимия, стадия, градация

Introduction

Liver cancer is currently the second most common cancer-associated cause of death worldwide [1]. Hepatocellular carcinoma (hepatoma, hepatocellular carcinoma), a malignant liver tumor originating from hepatocytes, accounts for up to 90% of all liver cancers [2].

In recent years, there has been an increase in the frequency of HCC throughout the world, for example, more than 600,000 newly diagnosed cases are recorded annually [3].

According to GLOBOCAN 2018, in the structure of oncopathology in terms of the incidence of HCC, it ranks 6th after lung and breast cancer, colorectal cancer, prostate and stomach cancer, and in terms of mortality it takes 4th place after lung cancer, colorectal cancer and cancer of stomach. However, in men, the incidence of morbidity and mortality from HCC is 2-3 times higher than in women, therefore, the incidence and mortality rates in men ranked 5th and 2nd, respectively [4, 5].

The etiology of HCC is multifactorial [6]. The main reasons for the development of HCC worldwide are chronic hepatitis (hepatitis B and C virus infections) and liver cirrhosis [7]. Aflatoxin B1 (AFB1) and chronic alcohol abuse may also be additional factors [8].

The highest HCC rates are observed in countries with economies in transition with a low human development index, for example, in some countries in Africa (Egypt, Gambia, Guinea) and East and Southeast Asia (Mongolia, Cambodia, and Vietnam). In Mongolia, the incidence of HCC is significantly higher than in any other country [4, 5].

Major risk factors vary by region. In regions with the highest risk of HCC (China, East Africa), chronic HBV infection and exposure to aflatoxin are the main determinants, while in other countries (Japan, Egypt), HCV infection is considered the predominant cause. In Mongolia, HBV and HCV infection, coinfection with HBV with HCV or HBV with a δ (delta) agent, as well as alcohol abuse, are the main risk factors for the development of HCC [4, 5].

Hepatocellular carcinoma is a serious medical and social problem in many countries of the world, including Kazakhstan. In recent years (2013 - 2017) in the Republic of Kazakhstan there has been an increase in the incidence of HCC to 5.5 cases per 100 thousand population, and the mortality rate remains at a high level (about 1000 people annually). In 2017, 82.3% of the observed HCC patients died by the end of the year. The five-year survival rate was 23.7% [9].

HCC is characterized by an aggressive course, in most cases, an unfavorable prognosis. The five-year survival rate for HCC does not exceed 18%, and the postoperative recurrence rate is about 50% [10].

In recent years, the immunohistochemical (IHC) research method has been widely used in the diagnosis of malignant neoplasms. IHC is an informative method not only in the differential diagnosis of HCC, but also in determining the degree of histological differentiation of cancer, which has a prognostic value in the course of the disease [11].

Alpha-fetoprotein (AFP) is a glycoprotein produced by fetal cells of the fetus in the fetal gastrointestinal tract, liver and yolk sac [12]. The reasons for the formation of AFP in liver cancer of adult patients have not yet been established. It is assumed that embryospecific cells appear in a malignant tumor with impaired intercellular-matrix interactions and a reduced level of differentiation of new generations of tumor cells, which resume the synthesis of AFP [13].

Since the 1970s, AFP has been used as a tumor marker for the diagnosis of HCC. An increase in the AFP level by more than 10 μg/l was noted in almost 75% of cases with HCC [14]. Serum AFP results are still considered the most important marker for the diagnosis of HCC today and, together with ultrasound techniques, can increase the diagnostic value. However, its values can be high in some non-malignant liver diseases (hepatitis, cirrhosis without HCC nodes), as well as it can be low in some patients with HCC [15].

In addition to the use of serum AFP and ultrasound as diagnostic tools, there are biological tumor markers of AFP in IHC that play an important role in the following aspects: monitoring of treatment outcomes, prognostic information, and detection of disease recurrence after removal [16].

The article analyzes the parameters of serum AFP, the expression of AFP-immunopositive cells depending on the stage and gradation of HCC, in addition, the degree of correlation between the values of the two methods is determined.

Purpose:

Comparative analysis of the level of AFP expression in IHC, depending on the stage and gradation of HCC, correlation analysis of serum AFP and the level of AFP expression of immunopositive HCC cells.

Materials and methods

A total of retrospectively analyzed data from 50 patients with HCC who underwent surgical treatment (resection, transplantation) at the Syzganov's NSCS in 2014 - 2019. There were 28 men, 22 women, aged 34 to 74 years (average age 49.7 ± 0.2 years). All patients underwent a serological blood test to determine the AFP level. The postoperative material was subjected to immunohistochemical examination to determine the area of staining, the degree and intensity of expression of AFP-immunopositive cells.

Immunohistochemical study

Paraffin sections were dewaxed and rehydrated according to a standard technique. The protocol included preheating to 65 °C, antigen recovery for 20 minutes at a temperature of 97 °C and further cooling to 65 °C. Then the slides were washed for 1-3 minutes with TBS-buffer (Dako), then staining was carried out in a Bio-Optica slide master, in manual mode with FLEX Polyclonal rabbit antibody by human Alpha-1-Fetoprotein. The Reveal Polyvalent HRP-DAB Detection System was used to visualize the immunohistochemical reaction. Sections were counterstained with Mayer's hematoxylin; Bio-Mount balm was used for the conclusion.

Evaluation of the expression of antigens in IHC studies was carried out according to generally

accepted methods. The intensity and area of staining was evaluated, the value of staining was determined. Negative expression was noted as 0; low expression, 1% -10% of the area was marked as «+», moderate expression, 10% -50% of the area - «++», and high expression, > 50% - «+++».

The statistical analysis was performed using Microsoft Excel 2007 software.

Results:

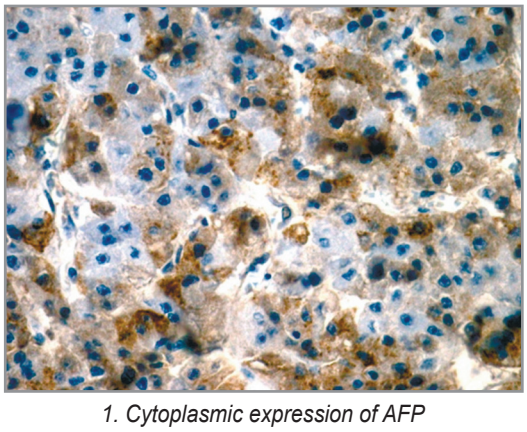
When analyzing the level of serological AFP, it was revealed that in the overwhelming majority of cases (n = 33) the AFP values were between 10-20 μg/l. At the same time, a critically high level of AFP (> 1000 μg/l) was found in 16% of cases (Table 1).

The results of the carried out immunohistochemical studies showed that in 83% of patients with HCC, cytoplasmic and nuclear expression of AFP is determined in malignant cells (Figure 1, 2).

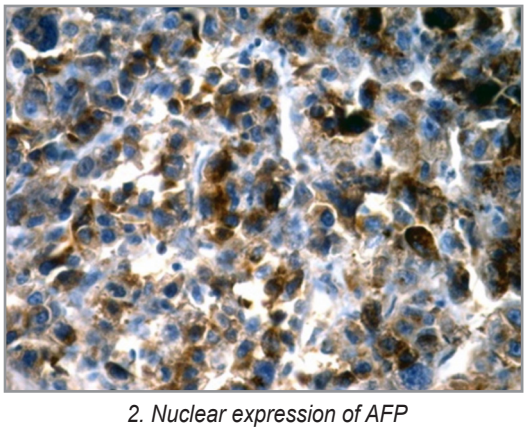
Table 1.
Distribution of patients with HCC by AFP level

AFP level, μg/l	HCC	
	n=50	%
10-20	33	66
20-100	3	6
100-200	3	6
200-500	1	2
500-1000	2	4
›1000	8	16

Figure 1,2.
HCC neoplastic cells with pronounced AFP expression. IHC x 200.



1. Cytoplasmic expression of AFP



2. Nuclear expression of AFP

To determine the characteristics of HCC, a comparative analysis was carried out according to the stages of the oncological process and the level of differentiation (gradation). Histological TNM classification was used to determine the stage of HCC. It was found that in most cases pT2 (34%), pT3 (32%), pT1 (16%) HCC stages were encountered in our sample. pT3 b and pT4 stages of the oncological process were detected in isolated cases. According to the degree of HCC differentiation (gradation), moderately differentiated HCC was detected in 46% (23) cases, low-differentiated HCC - in 30%, (15) cases, and highly differentiated HCC - in 24% (12) cases (Table 2).

Table 2.
Distribution of the HCC stage with the degree of differentiation (gradation)

Stage/ Gradation	n=50	n%	G1 n=12	G2 n=23	G3 n=15
pT1	8	16	8	-	-
pT2	17	34	2	12	3
pT3	16	32	2	6	8
pT3a	6	12	-	5	1
pT3b	2	4	-	-	2
pT4	1	2	-	-	1

The expression level of the AFP marker in the HCC node was high in 32% (n = 16) cases, moderate in 46% (n = 23) cases, and in 22% (n = 11) cases, the expression level of this hepatic glycoprotein was low or it was not determined at all (table 3). The area of AFP-immunopositive cells in hepatocellular liver cancer averaged 37.25 ± 15.47%. When analyzing the level of AFP expression depending on the HCC stage,

it was revealed that a high level of AFP expression was observed at pT2 - in 4% (2) cases, at pT3 and pT3a stages - 12% (6) cases in each, and at pT3b and pT4 stages - 2% (1) of cases in each. In most cases, moderate expression of AFP was observed at pT2 and pT3 stages of HCC (in 24% (12) and 20% (10) cases, respectively) (Table 3).

Table 3.
Expression level of AFP antigen at different stages of HCC

Expression Level/ Stage	pT1	pT2	pT3	pT3a	pT3b	pT4
Low	7	3	-	-	1	-
Moderate	1	12	10	-	-	-
High	-	2	6	6	1	1

Analyzing table 3, it can be revealed that high expression of AFP is observed in the HCC stages starting from pT2, pT3 and ending with pT4. Moderate expression of AFP is observed at stages pT2 and pT3 in relatively equal amounts. A low level of AFP can be seen mainly at the pT1 stage. Therefore, it can be assumed that the expression level increases from low to high depending on the stage of HCC (from pT2 to pT4). In total, it was revealed: in 46% of cases, moderate, in 32% of cases, high and in 22% - low expression of AFP. When analyzing the level of AFP depending on the gradation of HCC, it was found that, a low level of expression in 14% of cases out of 24% with highly differentiated HCC (G1 gradation), a moderate level of expression in 28% of cases out of 46% with moderately differentiated HCC (gradation G2), a high level of expression in 16% of cases out of 30% with low-differentiated HCC (gradation G3) (table 4).

Table 4.
Distribution of the AFP expression level by the level of HCC differentiation (gradation)

Expression level/ Gradation	n=50	n%	G1	G1%	G2	G2%	G3	G3%
Low	11	22	7	14	2	4	2	4
Moderate	23	46	4	8	14	28	5	10
High	16	32	1	2	7	14	8	16

Therefore, according to the data obtained, it can be concluded that the level of expression is directly proportional to the degree of HCC differentiation (gradation), i.e. with highly differentiated HCC (G1), the level of expression will be low, and with low-differentiated HCC (G3), the level of expression will be high. Correlation analysis revealed that the overall Pearson correlation coefficient between serum AFP and the degree of AFP staining on IHC was r = +0.0089, which corresponds to almost no correlation between these values (Figure 3). Accordingly, it can be assumed that serum AFP values will not be associated with AFP expression data during immunohistochemistry and can be used as a separate value for HCC differentiation.

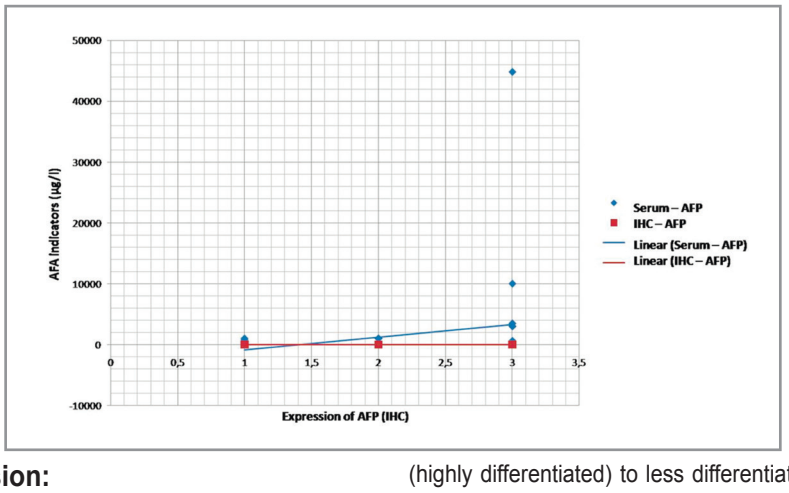


Figure 3.
Correlation of serum AFP parameters and expression of AFP-immunopositive cells

Conclusion:

Thus, in a comparative analysis of the level of alphafetoprotein expression with the stage and degree of HCC differentiation, the data obtained showed a relationship between these variables. A direct dependence of the AFP expression level on the HCC stage is noted; the expression level increases from pT1 to pT4. There is also an increase in the level of AFP expression from more differentiated HCC (highly differentiated) to less differentiated HCC (low-differentiated).

In our study the correlation analysis between serum AFP and the level of AFP expression in IHC revealed a weak correlation in the general cohort. It can be assumed that serum AFP values cannot be associated with data on AFP expression during immunohistochemistry and can be used as a separate value for HCC differentiation.

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