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Mucin expression in liver tumors

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Abstract---Difficulties in diagnosis, the prevalence of oncological processes, their medical, social and economic significance and high mortality rate determine the relevance of the fight against them and make the problem of studying the mechanisms of their early diagnosis one of the key ones. The subject of research now is the description of many biological markers, the determination of which allows us to assess the risks of developing a disease, diagnose a tumor, and serve as a biological indicator of the tumor process. The subjects of the study were tissue samples from 65 patients with liver cancer. The level of antibodies to MUC-1 and MUC-13 was studied. The established value of the concentration of antibodies to MUC-1 and MUC-13 in the blood serum of people with liver cancer is higher than the values in practically healthy individuals ($p = 0.002$; $p = 0.0001$), and the concentration of antibodies to MUC-1 and MUC-13 in the tissue tumors of cancer patients is higher than in the blood serum of healthy individuals and patients with liver cancer ($p < 0.05$). The expression level of MUC-1 and MUC-13 in the tumor tissue is not related to the age and sex of patients. Determination of antibodies to MUC-1 and MUC-13 in tissue can be recommended to control the completeness of tumor resection during surgical interventions.

Keywords---mucin, serum, tissue, cancer, liver.

Introduction

Difficulties in diagnosis, the prevalence of oncological processes, their medical, social, and economic significance, and a high level of mortality determine the urgency of combating them and make the problem of studying the mechanisms of their early diagnosis one of the key ones [1, 2]. In 2020, among the 19.3 million new cases of cancer in the world, there were changes in the structure of localization and frequency: breast cancer (11.7%), which surpassed lung cancer (11.4%) in detection, colorectal cancer (10%), prostate cancer (7.3%), stomach cancer (5.6%), and liver cancer (4.7%) [3, 4].

In 2020, 905,677 people fell ill with liver cancer (LC), and 830,180 patients died. Its prevalence rises with population aging, peaking at around the age of 70 years [5, 6]. The seriousness of the liver cancer problem lies in its rapid progression and extremely unfavorable prognosis: without treatment, the patient lives 2-6 months from the moment of diagnosis, and after surgical treatment, only a few survive to reach the 5-year milestone. High mortality is due to tumor resistance to chemotherapy, the development of serious complications associated with liver cirrhosis (LC), as well as late diagnosis, when curable methods of treatment become unavailable [7, 8].

Our country is included in the group of countries with a relatively low incidence of liver cirrhosis. About 35% of cases are diagnosed in stages III and IV. The five-year survival rate of patients, depending on the stage of the disease, varies from 60.6% to 14.5% [9]. The subject of research now is the description of many biological markers, the determination of which makes it possible to assess the risks of developing a disease, diagnose a tumor, and serve as a biological indicator of the tumor process [10, 11, 12]. High-molecular-weight glycoproteins, mucins (MUC), which are large O-glycoproteins with a high content of carbohydrates and a noticeable variety of both apoprotein and oligosaccharide fragments, have shown significant interest in themselves, from the point of view of biological activity [13].

Normally, in the human body, MUCs are expressed by cells of a single-layer epithelium, are localized on the apical surface of cells, and are part of a molecular system that contributes to the stability of the epithelial barrier in the event of damage. In their structure, MUCs contain tandem repeats of amino acids such as proline, threonine, and serine; it is on the last two that glycosylation occurs. In humans, 21 MUC genes have been identified, of which 15 are expressed in various regions of the gastrointestinal tract (GIT) [14, 15]. Based on their structure, function, and cellular localization, MUCs are divided into two classes: membranous (associated with the cell surface) and secreted [16]. Secreted MUC, including MUC2, MUC5AC, MUC5B, MUC6, and MUC7, create an impenetrable gel that forms a physical barrier and "detains" microorganisms and viruses [17].

In recent years, cell surface MUCs (membrane-bound, transmembrane MUC) have attracted more attention [18]. In the gastrointestinal tract, the MUCs of this subfamily are: MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17 [15]. The proportions of their expression depend on the localization in the gastrointestinal tract. For example, MUC1 ("polymorphic"

epithelial mucin) is mainly present in the stomach and pancreatic epithelium, MUC3 can be found in the intestine, and MUC4 ("tracheobronchial" mucin) is characteristic of the mouth and intestines [19]. Many areas of the gastrointestinal tract are capable of producing different types of MUCs, with the possibility of co-expression of more than one type of MUC by individual cells [20]. All MUCs in this group are expressed in the apical part of the epithelial cell membrane and usually have large extracellular domains. The general mechanisms of the transmembrane action of MUC are well known, especially MUC1 (the so-called cancer antigen 15-3 (CA 15-3) or (CD227), MUC4 and MUC16 (CA125), as well as their roles in oncogenesis and metastasis [13].

In malignant tumors, it is possible to detect an increased expression of MUC compared to normal epithelium, a change in their intracellular localization, and an increase in the content of hypoglycosylated forms of the glycoprotein, and MUC present on the surface membrane of tumor cells can be considered as an ideal target for targeted therapy [21, 22]. Clinical observations are supported by experimental studies, which suggest that MUC overexpression and its abnormal intracellular localization may increase the invasive and metastatic potential of malignant cells [23, 24].

Other studies have shown that the relationship between the MUC level and the clinical characteristics of the tumor is ambiguous: the expression of mucins is detected in cancer and characterized by the histological stage of the process. An increase in MUC in the blood is associated with the degree of differentiation, tumor size, and relation to the receptor [25]. In patients with a tumor process, MUC expression may be associated with the stage of the TNM tumor and the presence of metastases [26]. Many aspects of the potential role of mucin in the occurrence and progression of malignant tumors, as well as the possibility of practical application of the accumulated experimental and clinical data, remain insufficiently studied to date [27].

It is widely believed that hepatocellular carcinoma (HCC) does not produce MUCs, while cholangiocarcinoma (CC) or combined HCC-CC can produce these glycoproteins. However, a growing number of reports indicate that MUCs can be produced by HCC cells that do not display or have not yet undergone morphological differentiation into biliary phenotypes [13, 21]. It was shown that mRNA and MUC1 protein levels were significantly elevated in intrahepatic CC (ICC) tissue compared to paired non-tumor tissues. These data suggest that MUC1 promotes the progression of ICC through activation of the Wnt/ β -catenin pathway [28]. Many believe that the expression and localization of MUC1 proteins in primary liver carcinomas (PLC) can act as prognostic markers, and MUC1 molecules can be useful in differential diagnosis [29].

In the first study demonstrating the value of MUC13 as a biomarker in various carcinomas and other conditions, MUC13 was shown to be secreted by cancer cell lines, with the highest levels found in ovarian cancer cell lines. In addition, human serum levels of MUC13 were significantly elevated in patients with ovarian cancer, liver cancer, lung cancer, and other cancers, but not in patients with inflammatory bowel disease. The authors believe that the significance of the level of MUC13 in the blood serum and its relationship with tumor progression

requires further research [30]. This conclusion is consistent with the conclusions of other authors who showed that MUC13 overexpression is associated with the development of malignant neoplasms, in particular, in intrahepatic cholangiocarcinoma CC, when its level can be of therapeutic value, both as a prognostic marker and as a treatment goal [31]. Thus, MUC, as an object of study, is of interest for understanding the biology of malignant neoplasms and background processes preceding their development, as well as for improving methods of diagnosis and prognosis in oncological diseases.

The aim of the study was to evaluate the expression of high molecular weight glycoproteins MUC-1 and MUC-13 in liver tumors.

Materials and Methods

The objects of the study were tissue samples of 65 patients with a malignant process from the archives of the Grodno Regional Clinical Pathological Bureau and blood serum obtained when applying for advisory and medical assistance. The study was performed as part of a mandatory medical examination in accordance with the current protocols for diagnosis and treatment. The malignant nature of liver damage has been proven morphologically and is represented by the following options: HCC, nodular liver cancer, diffuse LR (histologically-HCC, CC). The age of the subjects at the time of diagnosis was 26-97 years; the median age (Me) - 62.6 ± 14.6 years, lower quartile (Q25) - 53 years; the upper quartile (Q75) - 73 years.

Among the examined persons there were 44 men (67.7%) and 21 women (32.3%). HCC of the liver was established in 27 cases (41.5%), CC-13 (20%), and nodular liver cancer, 25 (38.5%). Among the examined patients, 17 patients were diagnosed with cancer on the background of cirrhosis (13 men and 4 women). In 32.3% of cases (21 patients), distant metastases were noted (in the lung, lymph nodes, adrenal glands, peritoneum, spine). Blood samples from healthy people without malignant neoplasms or viral infections were collected as part of preventive studies on 34 people with an average age of 58.87.7 years (minimum 45 years, maximum 81 years), 19 men (55.9%), and 15 women (44.1%).

The study of the level of antibodies to MUC-1 and MUC-13 (ng/ml) was carried out using ELISA in tissue samples and blood serum of patients using a kit of reagents manufactured by Wuhan Fine Biological Technology Co. Ltd" (China) on the enzyme immunoassay analyzer "Mindray 96RA" (China). Serial sections were prepared from paraffin-embedded tissue samples. In accordance with the standard protocol, tissue samples were prepared for analysis with a set of reagents manufactured by MagneSil Genomic, Fixed System (Promega, USA).

Blood serum samples were obtained in a standard way using Vacuette vacuum systems with a coagulation activator manufactured by Greiner Bio-One, Austria. Preparation of blood samples for the study was carried out in a unified way: centrifugation (Fenox-24M centrifuge, China) at 3000g for 10 minutes. The results obtained during the study were entered into a database. Statistical data processing was carried out using the standard package of applied statistical programs, SPSS. The difference between the studied parameters was recognized

as significant at $p < 0.05$. Among the methods of mathematical processing used are:

- study of the type of distribution and obtaining numerical characteristics. In the case of a normal distribution, the variable was characterized using the mathematical expectation (mean) – M and the standard deviation ($\pm\sigma$). If the distribution of variables did not correspond to Gaussian, then the values of the upper (Q75) and lower quartiles (Q) and median (Me) were used to describe them;
- identifying the response to the impact in a two-sample task: with a normal distribution, to test the hypothesis of equality of the average values of the two groups of the variable, the Student's test (T) was used, if the distribution of the variable did not correspond to normal, the comparison of two independent groups of the studied variable was carried out using the Mann-Whitney test (U), and dependent groups - the Wilcoxon test (Z);
- the Hill method was used to compare shares (percentages);
- identification of the relationship between two variables with a normal distribution, the Pearson correlation coefficient (r) was used to assess the linearity of the relationship between the variables. If the distribution of the variables did not correspond to the normal distribution, Spearman's nonparametric correlation analysis (R) was used to assess the relationship between them.

Research results

The serum concentrations of antibodies to MUC-1 and MUC-13 receptors in healthy individuals and in blood samples from individuals with Liver Cancer are presented in table 1.

Table 1 - The concentration of the level of antibodies in the blood serum to the MUC-1 and MUC-13 receptors in healthy individuals and in patients with Liver Cancer

Group	Antibodies to the receptors	n	Average	Max	Min	σ	p
Healthy (p_k)	MUC-1 ng / ml (p_{1k})	34	0,250	0,11	0,48	0,10	p_{m1k-} $\sigma=0,002$
	MUC-13 ng / ml (p_{13k})	34	0,321	0,08	0,62	0,13	
Liver Cancer (p_o)	MUC-1 ng / ml (p_{1o})	38	0,381	0,26	0,52	0,06	p_{m13k-} $\sigma=0,0001$
	MUC-13, ng / ml (p_{13o})	38	0,940	0,45	1,90	0,39	

As shown in Table 1, in healthy individuals, the concentration of antibodies to the MUC-1 receptor was 0.250 ± 0.10 ng/ml and to the MUC-13 receptor – 0.321 ± 0.13 ng/ml, which corresponded to our previously obtained indicators in other age groups. The concentration of antibodies to MUC-1 and MUC-13 in the blood serum of patients with liver cancer had significant differences from the control figures: 0.381 ± 0.06 ng/ml ($p=0.002$) and 0.940 ± 0.39 ng/ml ($p=0,0001$), respectively.

It was important to determine the concentration of antibodies to mucins MUC-1 and MUC-13 in tissue samples, the results of which are presented in table 2.

Table 2-The concentration of the level of antibodies to MUC-1 and MUC-13 in the tumor tissue extract

Group	Antibodies to the receptors	n	Average	Max	Min	σ
Tumor (p _o)	MUC-1, ng / ml (p _{1o})	32	1,061	0,217	2,840	0,527
	MUC-13, ng / ml (p _{13o})	32	1,107	0,275	1,540	0,368

As can be seen from Table 2, the average concentration of antibodies to MUC-1 and MUC-13 in the liver tumor tissue extract was significantly higher than in the blood serum of practically healthy individuals and individuals with liver cancer, which is shown in Figure 1. Due to the insufficient number of samples of "healthy" liver tissue, the study in this group was not performed and, accordingly, a comparative assessment was not carried out. At the same time, the results of the concentration of MUC in serum samples of apparently healthy individuals and individuals with liver cancer were taken into account.

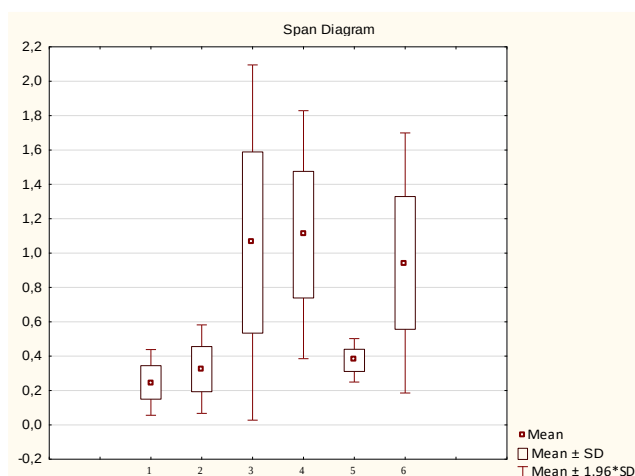


Figure 1. The concentration of the level of antibodies to MUC-1 and MUC-13 in the tumor tissue extract and in the serum of healthy individuals and individuals with liver cancer, abscissa: 1-serum of healthy individuals, MUC-1; 2-serum of healthy individuals, MUC-13; 3-liver tumor tissue, MUC-1; 4-liver tumor tissue, MUC-13; 5-serum of individuals with liver cancer, MUC-1; 6-serum of individuals with liver cancer, MUC-13; MUC concentration in ng/mL is shown on the y-axis.

The use of the Wilcoxon test for the analysis of two dependent groups made it possible to obtain the following Z-Test values and suggests that a significant concentration of antibodies to MUC-1 and MUC-13 in the tumor tissue is probably associated with resistance to apoptosis in RP, which requires further confirmation. research:

- MUC-1 (healthy, serum) & MUC-1 (tumor): $Z=4.843025$, $p=0.000001$;

- MUC-13 (healthy, serum) & MUC-13 (tumor): $Z=4.831024$, $p=0.000001$;
- MUC-1 (liver cancer, serum) & MUC-1 (tumor): $Z=4.674735$, $p=0.000003$;
- MUC-13 (liver cancer, serum) & MUC-13 (tumor): $Z=2.374765$, $p=0.017561$.

Since differentiation according to the TNM system (2010) is applicable only to HCC and CC, analysis of the dependence of MUC expression in tumor tissue on clinical data was not performed. An analysis of the relationship between the expression level of MUC-1 and MUC-13 in tumor tissue and the age of patients showed that there are no significant correlations between the levels of antibodies to MUC-1 and MUC-13 in the liver tissue extract affected by the tumor and the age of patients (Spearman, Median Test, Mann-Whitney U-Test): MUC-1 – $R = 0.053076$, $p = 0.772962$; MUC-13 – $R=0.190647$, $p=0.295940$). Thus, the level of antibodies does not depend on age, which increases its significance as a biological marker.

The distribution of concentrations (variables) of antibodies to MUC-1 and MUC-13 in the tissue extract in liver cancer within age groups (Q25–53 years, Q75–73 years) made it possible to estimate their concentrations with a minimum range (range of change) of the variable, the range of change for which will not be affected by the outlier, abnormal value (Figure 2).

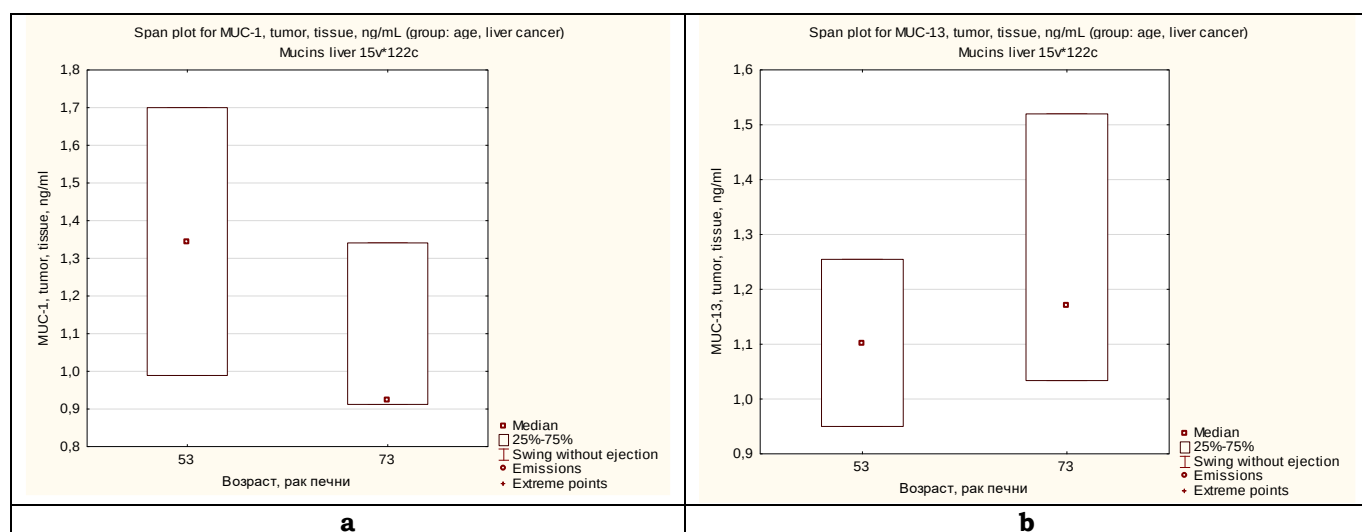


Figure 2. Diagram of the range of dependence of the concentrations of antibodies to mucins MUC-1 (a) and MUC-13 (b) in the tissue from the age of the patient.

Taking into account the range of variable expression of MUC-1 and MUC-13 in the age groups of persons diagnosed with liver cancer, in the age groups of 53 and 73 years, an assessment of multiple regression of the variables MUC-1 and MUC-13 and the predicted value of the concentration of MUC-1 and MUC-13 for persons with liver cancer (Figure 3).

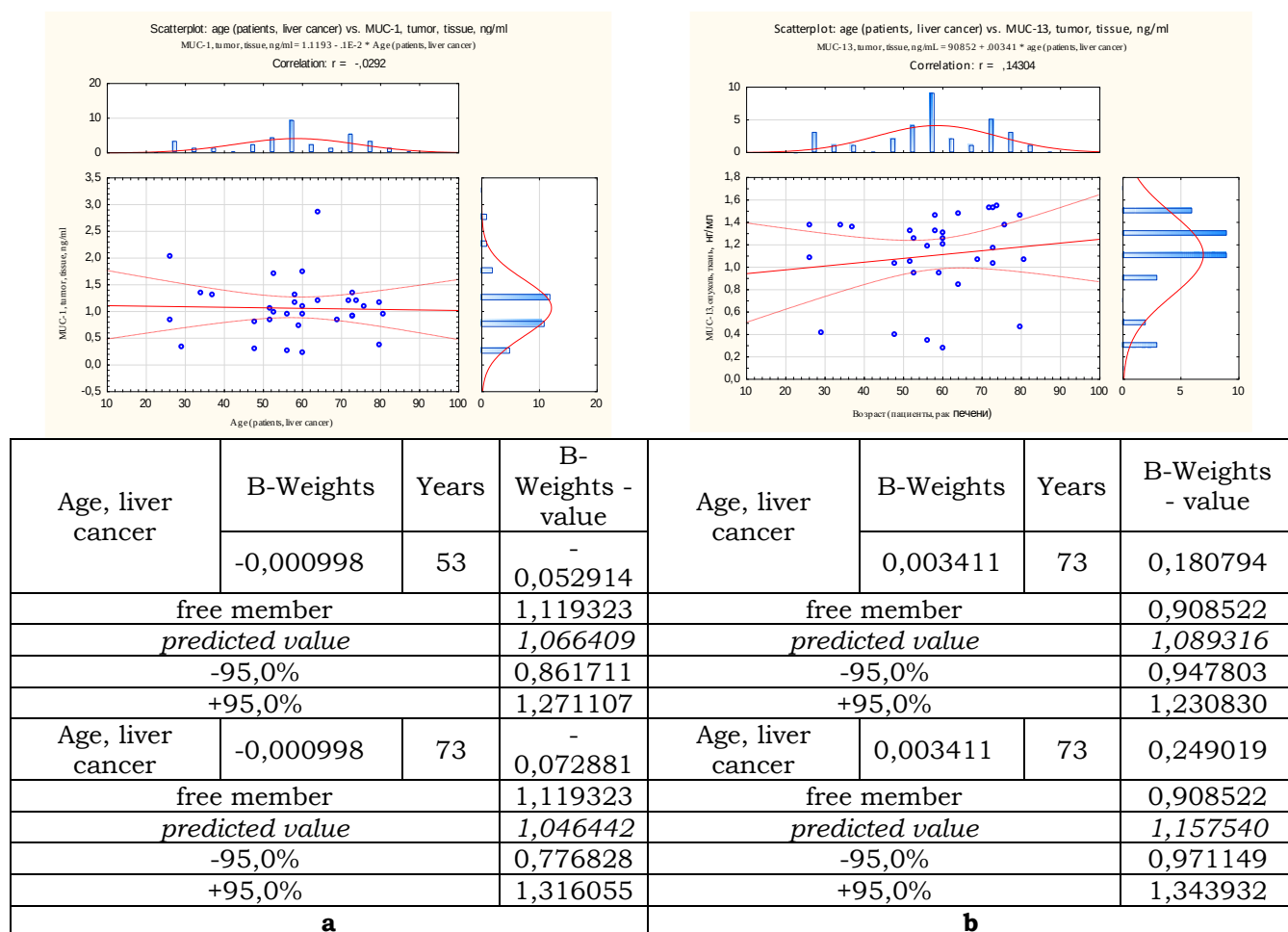


Figure 3. Multiple regression of the concentration of antibodies for the variables MUC-1 (a) and MUC-13 (b) in the tissue extract depending on the age of the patient, the predicted concentration value (ng/ml) for individuals with malignant liver processes

As shown by statistical analysis, the predicted value of the concentration of antibodies to MUC-1 and MUC-13 in tissue with liver cancer was: MUC-1 at 53 years old - 1.119 ng/ml, MUC-1 at 73 years old - 1.046 ng/ml, MUC-13 at 53 years old - 1.089 ng / ml, MUC-13 at 73 years old - 1.157 ng / ml. The estimated predicted value of MUC-1 and MUC-13 in serum for individuals with liver cancer is presented in Table 3.

Table 3 - The value of the concentration (predicted) of the level of antibodies to MUC-1 and MUC-13 in the blood serum of persons diagnosed with liver cancer

Mucin (MUC-1)	B-Beca	Year s	B-Weights - value	Mucin (MUC-13)	B-Weights	Years	B-Weights - value
Age, liver cancer	0,000345	53	0,018268	Age, liver cancer	0,000557	53	0,029500

free member			0,355145	free member			0,909718
<i>predicted value</i>			<i>0,373413</i>	<i>predicted value</i>			<i>0,939219</i>
-95,0%ИС			0,350236	-95,0%ИС			0,799962
+95,0%ИС			0,396590	+95,0%ИС			1,078475
Age, liver cancer	0,000345	73	0,025162	Age, liver cancer	0,000557	73	0,040632
free member			0,355145	free member			0,909718
<i>predicted value</i>			<i>0,380307</i>	<i>predicted value</i>			<i>0,950351</i>
-95,0%ИС			0,351017	-95,0%ИС			0,774369
+95,0%ИС			0,409596	+95,0%ИС			1,126333

The value of the concentration of antibodies to MUC-1 and MUC-13 in the blood serum in liver cancer was: MUC-1 at 53 years old, 0.373 ng/ml, MUC-1 at 73 years old, 0.380 ng/ml, MUC-13 at 53 years old, 0.939 ng/ml, MUC-13 at 73 years old, 0.950 ng/ml. The indicators of the predicted MUC value corresponded to the concentrations of these indicators in the MUC samples of patients with established liver cancer and did not differ significantly: MUC-1 (53.73 years) – $p=0.63$; MUC-13 (53.73 years) – $p=0.896$.

The relationship between the concentration of MUC in tissue and the gender of patients with liver cancer is shown in Figure 4.

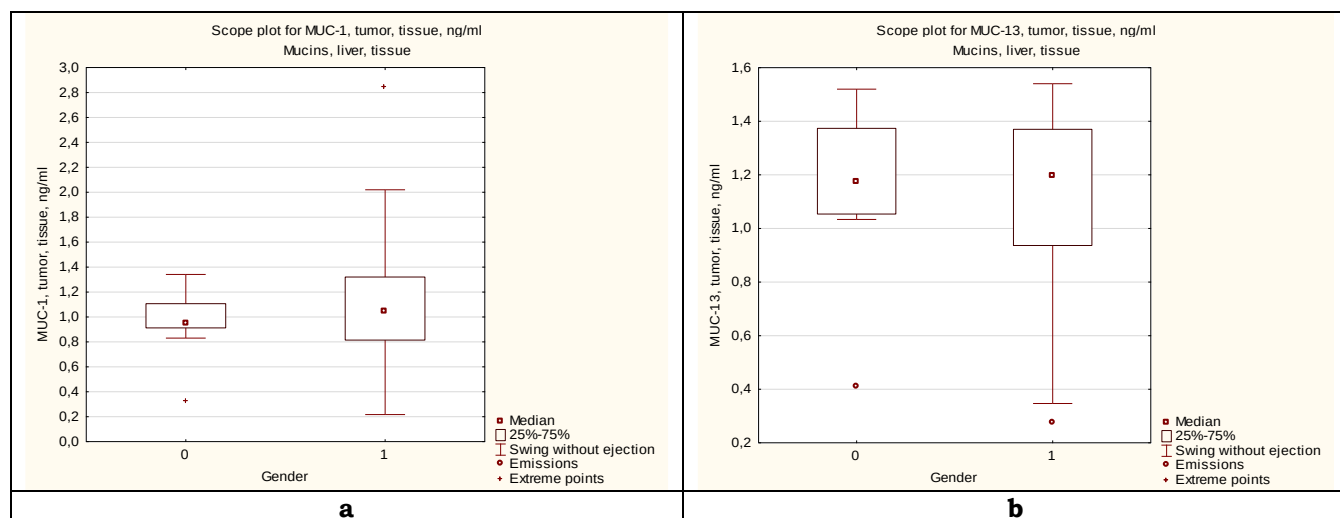


Figure 4. Dependence of antibody concentrations to MUC-1 (a) and MUC-13 (b) on patient gender (abscissa axis: 0 = female, 1 = male; y-axis: MUC-1/MUC-13 concentration, ng/ml)

No relationship was found between the level of MUC-1 and MUC-13 antibodies in the tissue and the gender of patients with liver cancer: MUC-1 - $R=0.086570$, $p=0.637559$, MUC-13 - $R=-0.060255$, $p=0.743219$ (Mann-Whitney U Test). Since the content of MUC-1 and MUC-13 antibodies in tissue did not correlate with the gender and age of patients, it is advisable to determine MUC-1 and MUC-13 in tissue in patients with any form of liver cancer, regardless of gender and age.

Determination of MUC in tissue can be recommended for use to control the completeness of the volume of tumor resection during surgical interventions.

Conclusion

Establishing the limits of the reference (normal) values of MUC-1 (0.250 ± 0.10 ng/ml) and MUC-13 (0.321 ± 0.13 ng/ml) in the blood serum of practically healthy individuals makes it possible to use these indicators as tumor markers for specifying for diagnosing liver cancer and for monitoring its progression. The concentration of antibodies to MUC-1 (0.381 ± 0.06 ng/ml, $p=0.002$) and MUC-13 (0.940 ± 0.39 ng/ml, $p=0.0001$) in blood serum in liver cancer was significantly higher than in practically healthy individuals. The concentration of MUC-1 and MUC-13 in the tumor tissue in liver cancer was significantly higher than in the blood serum of apparently healthy individuals and patients with liver cancer ($p<0.05$). The expression level of MUC-1 and MUC-13 in tumor tissue is not associated with age (MUC-1 – $p=0.77$; MUC-13 – $p=0.29$) and sex of patients (MUC-1 – $p=0.63$, MUC-13 – $p=0.74$).

Overexpression of MUC-1 and MUC-13 glycoproteins in tumor tissue may be associated with resistance to apoptosis, which needs to be confirmed. Determination of MUC-1 and MUC-13 in tissue can be recommended to control the completeness of the volume of tumor resection during surgical interventions. The MUC values are higher than the predicted concentrations in the liver tissue: MUC-1 – 1.066 ng/ml; MUC-13 – 1.089 ng / ml at 53 years of life of the patient and MUC-1 – 1.046 ng / ml and MUC-13 – 1.157 ng / ml at 73 years of age may indicate the risk of an occult tumor process.

The content of MUC-1 within the reference values (0.25 ± 0.10 ng/ml) indicates a low diagnostic value of this marker. With a confirmed diagnosis of liver cancer, the level of antibodies to MUC-1 in the blood serum exceeding 0.373 ng/ml and the level of antibodies to MUC-13 more than 0.939 ng/ml may indicate a high risk of a tumor process. MUC-1 and MUC-13 as a serological marker in patients with liver cancer is of interest for studying the biological characteristics of tumors, as well as for searching and developing additional criteria for clarifying the diagnosis and prognosis of the disease.

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