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Role of Serum and Ultrasonometric Markers in the Early Diagnosis of Malignant Transformation of Periductal Liver Fibrosis in Patients with *Opisthorchis Felineus* Infection

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Aim: to study the role of serum and ultrasonometric markers in the early diagnosis of malignant transformation of periductal fibrosis of the liver during the formation of cholangiocarcinoma against the background of chronic invasion of *Opisthorchis felineus*, in the population of endemic regions.
Methods. A comprehensive one-stage comparative study was conducted in a case-control design, using the assessment of biochemical parameters (lactate, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, glucose, direct and indirect bilirubin, high-density lipoproteins and low-density lipoproteins, triglycerides, urea and total protein), immunological parameters (transforming growth factor beta) and tumor markers (CA 19-9, CYFRA 21-1) in blood serum samples obtained from patients with *O. felineus* infection, with and without periductal fibrosis, *O. felineus*-infected patients with cholangiocarcinoma and representatives of control groups. During the analytical stage, the relationship between the structural features of the liver according to the results of ultrasound examination (presence of opisthorchiasis-associated periductal fibrosis), clinical parameters, parasitological study data (infection intensity), and changes in biochemical, immunological parameters and tumor markers in patients against the background of *O. felineus* infection was assessed. Statistical analysis was performed using the R programming language (version 3.6.1).
Results. Evaluation of the relationship between the presence of ultrasound signs of stage 1–2 opisthorchiasis-associated periductal fibrosis and changes in serum levels of the biochemical, immunologic parameters and oncomarkers in patients with *O. felineus* infection demonstrated that the levels of alkaline phosphatase, conjugated bilirubin, AST, glucose and CA 19-9 oncomarker were significantly higher in the serum of patients in the group with cholangiocarcinoma and opisthorchiasis ($p < 0.01$).
Conclusion. The first pilot data on the relationship between ultrasonometric signs of periductal liver fibrosis and the increase of CA 19-9 oncomarker as well as biochemical parameters (alkaline phosphatase, direct bilirubin, aspartate aminotransferase, glucose), which can be used for early diagnosis of malignant transformation of periductal liver fibrosis with the formation of cholangiocarcinoma in *O. felineus*-infected population in endemic regions, were obtained.
Keywords: cholangiocarcinoma, tumor markers, cancer, periductal fibrosis, lactate, biochemical markers, *Opisthorchis felineus* infection, ultrasonography
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Роль сывороточных и ультрасонометрических маркеров в ранней диагностике злокачественной трансформации перидуктального фиброза печени на фоне инвазии *Opisthorchis felineus*

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Цель исследования: изучить роль сывороточных и ультрасонометрических маркеров в ранней диагностике злокачественной трансформации перидуктального фиброза печени при формировании холангиокарциномы на фоне хронической инвазии *Opisthorchis felineus* у населения эндемичных регионов.
Методы. Проведено комплексное одномоментное сравнительное исследование в дизайне «случай-контроль» с использованием оценки биохимических параметров (лактат, аланинаминотрансфераза, аспартатаминотрансфераза, гамма-глутамилтранспептидаза, щелочная фосфатаза, глюкоза, прямой и непрямой билирубин, липопротеины высокой плотности и липопротеины низкой плотности, триглицериды, мочевина и общий белок), иммунологических параметров (трансформирующий фактор роста бета 1) и онкомаркеров (CA 19-9, CYFRA 21-1) в образцах сыворотки крови, полученных от больных инвазией *O. felineus*, имеющих перидуктальный фиброз и без такового, больных холангиокарциномой, развившейся на фоне инвазии *Opisthorchis felineus*, и больных описторхозом, а также представителей группы контроля.
Результаты. Оценка взаимосвязи между наличием ультразвуковых признаков описторхоз-ассоциированного перидуктального фиброза 1–2-й степени и изменениями биохимических, иммунологических параметров и содержания онкомаркеров у пациентов на фоне инвазии *O. felineus* продемонстрировала, что уровни щелочной фосфатазы, прямого билирубина, АСТ, глюкозы и онкомаркера CA 19-9 были значимо выше в группе пациентов с холангиокарциномой на фоне описторхоза, чем у исследуемых групп с перидуктальным фиброзом на фоне инвазии и без нее, а также контролями ($p < 0,01$).
Выводы. Впервые получены пилотные данные о взаимосвязи ультрасонометрических признаков перидуктального фиброза печени и увеличения биохимических показателей (щелочная фосфатаза, прямой билирубин, аспартатаминотрансфераза, глюкоза), а также повышения онкомаркера CA 19-9, которые могут быть использованы для ранней диагностики злокачественной трансформации перидуктального фиброза печени с формированием холангиокарциномы на фоне хронической инвазии *O. felineus* у населения эндемичных регионов.
Ключевые слова: холангиокарцинома, онкомаркеры, рак, перидуктальный фиброз, лактат, биохимические маркеры, инвазия *Opisthorchis felineus*, ультрасонография
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Introduction

Liver cancer is a socially significant pathology in the world foci of human trematodiasis [1–8]. Cholangiocellular carcinoma (cholangiocarcinoma, CCA) is one of the most unfavorable malignant neoplasms in terms of prognosis due to high mortality, the aggressiveness of tumors, lack of specific symptoms, long asymptomatic course and resistance to therapy [9, 10].

At present there is enough epidemiologic data confirming the correlation of CCA development with hepatic trematodiasis, widespread in Southeast Asia. Thus, trematodes *Opisthorchis viverrini* (*O. viverrini*) and *Clonorchis sinensis* (*C. sinensis*) are included in the register of biological carcinogens of group 1 with proven oncogenicity for humans [11, 12].

In endemic regions of Russia, the study of the problem of opisthorchiasis and associated chronic diseases is of relevance, since the liver trematode *Opisthorchis felineus* (*O. felineus*) is the cause of chronic inflammatory process, which is asymptomatic for a long time and contributes to the development of periductal fibrosis, predisposing to the development of CCA [5, 13, 14]. Previously published epidemiologic data demonstrated a significant association of *O. felineus* infection with liver fibrosis and CCA [15, 16].

Currently, no algorithms for early preclinical diagnosis of CCA associated with chronic infection of *O. felineus* have been developed in the world, which leads to late verification of the diagnosis, untimely treatment, and high mortality [17, 18].

Conducting a study to develop new approaches to screening of oncologic diseases of the hepatobiliary zone will improve early diagnosis of severe liver diseases, conduct timely treatment of patients, contributing to improved prognosis in this category of patients.

Thus, the aim of the study is to investigate the role of serum and ultrasound markers in early diagnosis of malignant transformation

of periductal liver fibrosis in the formation of cholangiocarcinoma in *O. felineus*-infected population of the endemic regions.

Materials and methods

The study was performed on the basis of the Siberian State Medical University (SibMed); the protocol was approved by the Ethical Committee of SibMed (conclusion No. 4815 of 20.06.2016). A one-stage comparative case-control study was performed.

The study recruited participants of the previously conducted projects "Fundamental approaches to optimize the diagnosis and therapy of natural-focal infection of *Opisthorchis felineus* in endemic regions" and "Identification of new diagnostic markers for the development of technologies for population screening of trematodiasis" [14, 15]. The following study groups of adult patients were formed:

– Group 1 — patients with histologically verified CCA and opisthorchiasis (n = 24);

– Group 2 — patients with *O. felineus* infection complicated by periductal fibrosis according to ultrasound examination (US) of the hepatobiliary system (n = 36);

– Group 3 — patients with *O. felineus* infection and without periductal fibrosis according to ultrasound of the hepatobiliary system (n = 29);

– Group 4 — a control group consisting of volunteers without *O. felineus* infection and without clinically significant changes in the hepatobiliary system (n = 30).

The inclusion criterion for participants in all groups was the presence of signed informed consent for participation in the study. The diagnosis of "cholangiocarcinoma" was made based on the results of histologic and/or histochemical examination of biopsy or postoperative liver material, according to the following ICD-10 codes: C22.1 — cancer of the intrahepatic bile duct; C24 — malignant neoplasm of other and unspecified parts of biliary tract; C24.0 — malignant neoplasm of extrahepatic bile duct; C24.9 — malignant neoplasm of biliary tract unspecified.

O. felineus infection was diagnosed by a positive result of parasitological examination (microscopy of two stool samples using Parasep concentrators (DiaSys Ltd., UK)).

The presence of periductal fibrosis of the 1–2nd degree was confirmed by ultrasound of the hepatobiliary system using a high-resolution mobile ultrasound scanner ("Mindray M7", "Shenzhen Mindray Bio-Medical Electronics Co., Ltd.", China) in accordance with the protocol proposed within the framework of epidemiologic studies of opisthorchiasis previously conducted in Southeast Asia [19]. The liver size and structural features of the liver parenchyma (echo signs of periductal fibrosis: increased periportal echogenicity of more than 3 mm around the intrahepatic bile ducts of the second order; as well as dilation and thickening of the bile duct wall) were evaluated.

Biochemical study of blood serum was performed at the central clinical and diagnostic laboratory of SibMed using an automatic biochemical analyzer ARCHITECT c4000 ("Abbot", USA) with quantitative determination of the following parameters: aspartate aminotransferase (AST); alanine aminotransferase (ALT); total, direct and indirect bilirubin; total albumin, total cholesterol; high density lipoproteins (HDL); low-density lipoproteins (LDL); triglycerides; urea; gamma-glutamyl transpeptidase (GGT); alkaline phosphatase (ALP), glucose ("DiaSys Diagnostic Systems GmbH", Germany). Lactate concentration in serum was measured using Biosen C-line analyzer with built-in chip-sensor ("EKF-diagnostic GmbH", Germany).

Immunologic methods of determining the level of cytokine transforming growth factor beta 1 (TGF-β1), as well as CA 19-9 and CYFRA 21-1 oncomarkers in serum were performed by enzyme immunoassay using kits from Chema LLC (Russia) and Cloud-Clone Corp. (USA).

Statistical analysis of the data was performed using the R programming language version 3.6.1. To assess intergroup differences in the measured parameters, the Kraskell — Wallis test and the Mann — Whitney — Wilcoxon test were used in the absence of normal distribution, or ANOVA and Student's t-test in the presence of normal distribution. The methods were implemented in the stats package (R Core Team, 2019). The distributions of parameters were checked for conformity to the normal law using the Shapiro — Wilk criterion. Relationships were determined using Spearman rank correlation implemented in the package "psych" (R package version 1.9.12). Categorical variables were compared using Pearson's χ^2 test.

The "ggplot2" package (2016) was used for visualization. Additional identification of factors associated with periductal fibrosis was performed using the Boruta method. This method is based on the use of a random forest algorithm to classify observations according to the measured parameters in accordance with a given grouping (by the presence of CCA, periductal fibrosis, *O. felineus* infection) and the subsequent assessment of the importance of each parameter for classification [20].

Results

One hundred and nineteen participants were included in the study: 53.0 % were male; age — 18–87 years; mean age — 53.1 ± 1.6 years. The groups were comparable by sex and age. Demographic and clinical characteristics of the study sample are presented in Table 1.

In the comparison groups (Groups 1–3), the mean infection intensity was evaluated and it was 1284.1 ± 655.6, 2083.3 ± 760.5 and 865.6 ± 406.3 helminth eggs in 1 g of stool, respectively (Table 1).

The results demonstrated that the symptoms such as jaundice, abdominal pain, hepatomegaly, liver tenderness on palpation, and bladder symptoms were predominantly found in CCA patients with opisthorchiasis (Group 1). Most of the patients suffering from *O. felineus* infection (Groups 1, 2 and 3) had clinically distinct form of the disease. The most common symptoms in the study participants were abdominal pain, bloating, positive bladder symptoms of Ker and Ortner, liver tenderness on palpation, and hepatomegaly (Table 2).

According to the results of ultrasound of the hepatobiliary system, hepatomegaly, neoplasms

Table 1. Demographic and clinical characteristics of the study groups
Таблица 1. Демографические и клинические характеристики исследуемых групп

Parameter / *Показатель*	Group 1 *Группа 1*	Group 2 *Группа 2*	Group 3 *Группа 3*	Group 4 *Группа 4*	*p*
Demographic characteristics / *Демографические характеристики*					
Gender. *n* (%) *Пол. n (%)* male / *мужской* female / *женский*	 15 (62.5) 9 (37.5)	 17 (47.2) 19 (52.8)	 16 (55.2) 29 (44.8)	 15 (50) 15 (50)	$p > 0.05^*$
Age. years *Возраст. лет* 95% ДИ / 95 % CI *M ± m*	 63.0 ± 2.2 8.6–15.4	 54.1 ± 2.4 11.4–18.4	 48.9 ± 3.5 15.03–25.6	 48 ± 3.4 14.9–25.2	$p > 0.05^*$
Physical examination / *Физикальное исследование*					
Fever. *n* (%) *Лихорадка. n (%)* yes / *да* no / *нет*	 1 (4.2) 23 (95.8)	 2 (5.6) 34 (94.4)	 1 (3.4) 28 (96.7)	 –	$p_{1-2} > 0.05$ $p_{1-3} > 0.05$ $p_{2-3} > 0.05$
Jaundice. *n (%)* *Желтуха. n (%)* yes / *да* no / *нет*	 7 (29.2) 17 (70.8)	 1 (2.8) 35 (97.2)	 –	 –	$p_{1-2} < 0.05$
Abdominal pain. *n (%)* *Боль в животе. n (%)* yes / *да* no / *нет*	 12 (50.0) 12 (50.0)	 12 (33.3) 36 (66.7)	 1 (3.4) 28 (96.7)	 –	$p_{1-2} < 0.05$ $p_{1-3} < 0.05$ $p_{2-3} < 0.05$
Bloating. *n (%)* *Вздутие живота. n (%)* yes / *да* no / *нет*	 2 (8.3) 22 (91.7)	 12 (33.3) 24 (66.7)	 1 (3.4) 29 (96.6)	 3 (10.0) 27 (90.0)	$p_{1-2} < 0.05$ $p_{2-3} < 0.05$ $p_{2-4} < 0.05$
Positive signs of gallbladder disease. *n (%)* *Положительные пузырные симптомы. n (%)* yes / *да* no / *нет*	 12 (50.0) 12 (50.0)	 2 (5.6) 34 (94.4)	 –	 –	$p_{1-2} < 0.05$

Continuation of Table 1. Demographic and clinical characteristics of the study groups
Продолжение таблица 1. Демографические и клинические характеристики исследуемых групп

Microscopy of stool samples / *Микроскопия образцов стула*					
Intensity of *O. felineus* infestation (eggs per 1 g of stool) *Интенсивность инвазии O. felineus (яиц в 1 г стула)* *M ± m* *95% ДИ / 95 % CI*	1284.1 ± 655.6 1858.8–4004.2	2083.3 ± 760.5 3639.1–5894.6	865.6 ± 406.3 1466.2–2723.5	–	$p > 0.05^*$
Ultrasound examination of the hepatobiliary system / *Ультразвуковое исследование гепатобилиарной системы*					
Dilation of bile ducts, *n* (%) *Расширение желчных протоков, n (%)* yes / *да* no / *нет*	13 (54.2) 11 (42.8)	3 (8.3) 33 (91.7)	2 (6.9) 27 (93.1)	–	$p_{1–2} < 0.05$ $p_{1–3} < 0.05$ $p_{1–4} < 0.05$
Thickened, uneven walls of bile ducts, *n* (%) *Утолщение, неровные стенки желчных протоков, n (%)* yes / *да* no / *нет*	10 (41.7) 14 (58.3)	4 (11.1) 32 (88.9)	1 (3.4) 28 (96.6)	1 (3.3) 29 (96.7)	$p_{1–2} < 0.05$ $p_{1–3} < 0.05$ $p_{1–4} < 0.05$
Periductal fibrosis, *n* (%) *Перидуктальный фиброз, n (%)* yes / *да* no / *нет*	18 (75.0) 6 (25.0)	36 (100) 0 (0)	–	–	$p_{1–2} < 0.05$

Note: *M ± m* — mean ± standard error; 95 % CI — 95 % confidence interval; * — in all groups for pairwise comparison, $p_{1–2}$ — for comparison of groups 1 and 2; $p_{1–3}$ — when comparing groups 1 and 3; $p_{2–3}$ — when comparing groups 2 and 3; $p_{1–4}$ — when comparing groups 1 and 4; $p_{2–4}$ — when comparing groups 2 and 4.
Примечание: *M ± m* — среднее ± стандартная ошибка; 95 % ДИ — 95 %-ный доверительный интервал; * — во всех группах при попарном сравнении, $p_{1–2}$ — при сравнении групп 1 и 2; $p_{1–3}$ — при сравнении групп 1 и 3; $p_{2–3}$ — при сравнении групп 2 и 3; $p_{1–4}$ — при сравнении групп 1 и 4; $p_{2–4}$ — при сравнении групп 2 и 4.

in the liver, thickening and irregularity of the gallbladder wall, cholelithiasis, periductal fibrosis, as well as dilation and structural changes of the bile ducts were statistically significantly higher in Group 1. Patients in Group 2 were also significantly more likely to have liver enlargement and biliary tract pathology ($p < 0.05$). The presence of masses in the liver was verified by computerized and/or magnetic resonance imaging in all participants from Group 1 (Table 1).

The study also evaluated the levels of biochemical markers (lactate, AST, ALT, GGT, alkaline phosphatase, glucose, direct and indirect bilirubin, HDL, LDL, triglycerides, total cholesterol, urea, and total protein), as well as immunologic markers (TGF-β1) and oncomarkers (CA 19-9, CYFRA 21-1) in serum samples obtained from patients with *O. felineus* infection having CCA and periductal fibrosis (Groups 1 and 2, respectively), from patients without CCA and periductal fibrosis (Group 3) and from uninfected individuals without malignancy (Group 4). The levels of alkaline phosphatase, direct bilirubin, AST and glucose were significantly higher in patients with CCA and *O. felineus* infection compared to other groups in pairwise comparison ($p < 0.01$). The results of the study of blood serum samples are presented in Tables 2–3.

Evaluation of the relationship between the peculiarities of the liver structure according to the results of ultrasound examination (presence of opisthorchiasis-associated stage 1–2 periductal fibrosis and changes in biochemical, immunologic parameters and content of oncomarkers in serum in patients against the background of *O. felineus* infection demonstrated that the level of CA 19-9 in the group of patients with CCA was significantly higher compared to patients of other groups ($p < 0.01$). In Groups 2–4, no significant differences in the levels of the studied oncomarkers were obtained.

Visualization method using the package “ggplot2” (Fig. 1) showed the significant increase in such biochemical parameters as alkaline phosphatase, conjugated bilirubin, AST, glucose, as well as high CA 19-9 oncomarker in patients in ;Group 1 with CCA and opisthorchiasis ($p < 0.01$).

The x-axis shows the measured factors on which the classification of patients into clinical

Table 2. Results of determination of biochemical parameters in the study groups, *Me (Q1; Q3)**
Таблица 2. Результаты определения биохимических показателей в исследуемых группах, *Me (Q1; Q3)**

Parameter / *Показатель*	Group 1 *Группа 1* (CCA+ OPI+)	Group 2 *Группа 2* (PF+ OPI+)	Group 3 *Группа 3* (PF– OPI+)	Group 4 *Группа 4* (PF– OPI–)
AST, U/L *АСТ, Ед./л*	42.5 (30; 78.5)	19.0 (15; 23.5)	16.5 (14.0; 31.3)	19.0 (14.0; 31.3)
ALT, U/L *АЛТ, Ед./л*	23.5 (9.5; 57.5)	9.5 (6.0; 17.8)	10.0 (6.0; 17.0)	10.0 (6.0; 19.0)
Total bilirubin, umol/L *Билирубин общий, мкмоль/л*	16.5 (6.8; 77.4)	5.2 (3.2; 9.4)	7.3 (3.8; 12.4)	10.1 (1.9; 15.4)
Direct bilirubin, umol/L *Билирубин прямой, мкмоль/л*	5.3 (2.6; 39.3)	2.4 (1.9; 3.1)	2.6 (1.9; 3.6)	2.35 (1.8; 3.8)
Total protein, g/L Общий белок, г/л	70.0 (59.8; 78.3)	80.0 (75.0; 84.0)	80.0 (72.5; 83.0)	75.5 (69.9; 80.9)
Albumin, g/L *Альбумин, г/л*	33.5 (28; 43)	45.0 (20.7; 48.0)	47.0 (43.0; 48.5)	46.0 (14.1; 48.5)
Total cholesterol, mmol/l *Общий холестерол, ммоль/л*	4.9 (3.9; 5.7)	5.4 (4.6; 5.7)	4.6 (4.2; 5.4)	4.2 (3.5; 5.0)
HDL, mmol/L *ЛПВП, ммоль/л*	0.9 (0.6; 1.3)	1.3 (1.1; 1.7)	1.3 (1.2; 1.8)	1.4 (1.0; 1.8)
Triglycerides, mmol/L *Триглицериды, ммоль/л*	1.3 (0.9; 1.6)	1.0 (0.7; 1.6)	0.9 (0.7; 1.2)	0.6 (0.4; 0.9)
Urea, mmol/L *Мочевина, ммоль/л*	4.9 (4.2; 8.9)	5.1 (3.9; 6.6)	3.9 (3.2; 5.7)	4.5 (4.2; 4.8)
GGT, U/L *ГГТ, Ед./л*	250.5 (144.0; 715.5)	20.0 (16.0; 29.0)	22.0 (13.3; 60.0)	14.0 (11.8; 30.3)
ALP, U/L *ЩФ, Ед./л*	216.0 (145.5; 429.0)	75.0 (65.0; 95.0)	70.0 (47.8; 80.0)	66.0 (54.0; 79.0)
Glucose, mmol/L *Глюкоза, ммоль/л*	5.6 (4.9; 7.2)	4.8 (4.1; 5.2)	4.5 (4.2; 4.9)	4.8 (4.0; 7.6)
Lactate, mmol/L *Лактат, ммоль/л*	3.1 (2.5; 3.5)	4.2 (3.0; 5.5)	4.05 (2.6; 5.5)	4.1 (2.6; 5.2)

Note: * — data are presented as median (*Me*), upper (*Q1*) and lower (*Q3*) quartiles; CCA — cholangiocarcinoma; OPI — *O. felineus* infection; PF — periductal fibrosis; AST — aspartate aminotransferase; ALT — alanine aminotransferase; HDL — high density lipoproteins; GGT — gamma glutamyl transpeptidase; ALP — alkaline phosphatase; "–" or "+" — presence or absence of a sign.
Примечание: * — данные представлены в виде медианы (*Me*), верхнего (*Q1*) и нижнего (*Q3*) квартилей; ССА — холангиокарцинома, ОРІ — инвазия *O. felineus*; PF — перидуктальный фиброз; АСТ — аспартатаминотрансфераза; АЛТ — аланинаминотрансфераза; ЛПВП — липопротеины высокой плотности; ГГТ — гамма-глутамилтрансфераза; ЩФ — щелочная фосфатаза; «–» или «+» — наличие или отсутствие признака.

Table 3. Results of determination of immunological and tumor markers by ELISA in the study groups, *Me (Q1; Q3)**
Таблица 3. Результаты определения иммунологических и онкомаркеров методом ИФА в исследуемых группах, *Me (Q1; Q3)**

Parameter / *Показатель*	Group 1 *Группа 1* (CCA+ OPI+)	Group 2 *Группа 2* (PF+ OPI+)	Group 3 *Группа 3* (PF– OPI+)	Group 4 *Группа 4* (PF– OPI–)
TGF-β1, pg/mL / *пг/мл*	573.0 (308.3; 783.0)	498.0 (262.5; 649.5)	498.0 (349.5; 675.0)	507.0 (351.8; 669.8)
CA 19-9, U/L / *Ед./мл*	32.3 (14.8; 240.0)	8.4 (4.1; 12.1)	6.0 (3.6; 12.8)	8.0 (5.0; 16.5)
CYFRA 21-1, ng/mL / *нг/мл*	2.0 (0.9; 3.8)	0.9 (0.5; 2.1)	0.9 (0.8; 1.8)	0.9 (0.3; 1.7)

Note: * — data are presented as median (*Me*), upper (*Q1*) and lower (*Q3*) quartiles; CCA — cholangiocarcinoma; OPI — *O. felineus* invasion; PF — periductal fibrosis; "–" or "+" — presence or absence of a sign; TGF-β1 — transforming growth factor beta 1; CA 19-9, CYFRA 21-1 — oncomarkers.
Примечание: * — данные представлены в виде медианы (*Me*), верхнего (*Q1*) и нижнего (*Q3*) квартилей; ССА — холангиокарцинома, ОРІ — инвазия *O. felineus*; PF — перидуктальный фиброз; «–» или «+» — наличие или отсутствие признака; TGF-β1 — трансформирующий фактор роста бета 1; CA 19-9, CYFRA 21-1 — онкомаркеры.

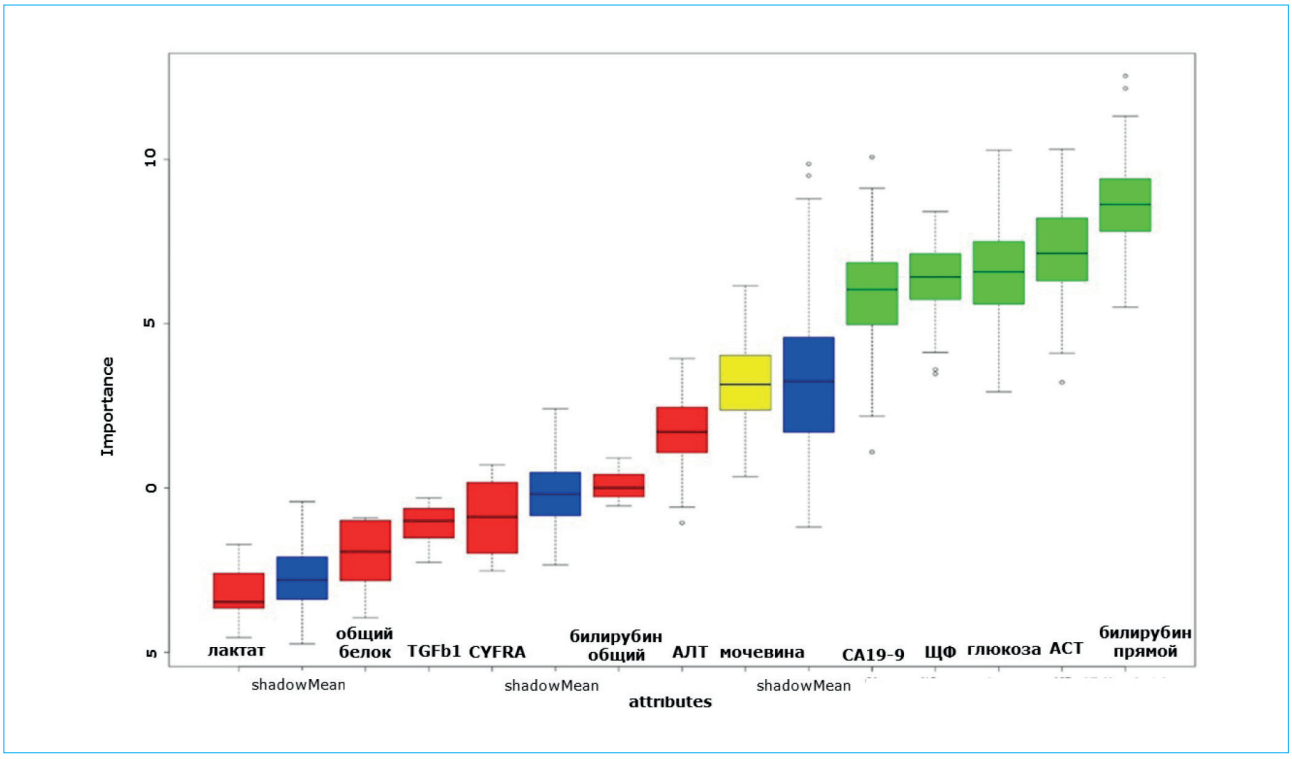

Figure. Estimation of the importance parameter of biochemical parameters and tumor markers for predicting opisthorchiasis-associated periductal fibrosis (using the ggplot2 package); ALP — alkaline phosphatase; ALP — alkaline phosphatase; ALT — alanine aminotransferase; AST — aspartate aminotransferase; TGF-β1 — transforming growth factor beta 1; CA 19-9, CYFRA 21-1 — oncomarkers

Рисунок. Оценка параметра важности биохимических показателей и онкомаркеров для предсказания описторхоз-ассоциированного перидуктального фиброза (с использованием пакета «ggplot2»); АЛТ — аланинаминотрансфераза, АСТ — аспартатаминотрансфераза, ЩФ — щелочная фосфатаза, TGF-β1 — трансформирующий фактор роста бета 1; CA 19-9, CYFRA 21-1 — онкомаркеры

groups was performed, among them shadowMin, shadowMean and shadowMax are the artificial factors used to assess the reliability of importance, the y-axis shows the importance of each factor for classification. Green color indicates factors for which importance significantly ($p < 0.05$) exceeded the importance of artificial factors entered into the model, blue color indicates artificial factors, red and yellow colors indicate factors with importance lower than or equal to artificial factors.

When analyzing the association between the level of lactate and biochemical parameters, immunological, oncological markers in serum and the intensity of *O. felineus* infection, it was found that in the group of patients with *O. felineus* infection complicated by periductal fibrosis there is a tendency to decrease CA 19-9 oncomarker in participants with higher intensity of *O. felineus* infection ($p = 0.05$).

As a result of this study, pilot data on the relationship between ultrasonometric signs of increased periportal and periductal echogenicity and increased biochemical parameters (alkaline phosphatase, direct bilirubin, AST, glucose) and CA 19-9 oncomarker, which can serve as early markers of malignant transformation of periductal fibrosis *O. felineus*-infected population in endemic regions, were obtained.

Discussion

The problem of hepatobiliary system diseases associated with chronic opisthorchiasis is still relevant in regions of the Russian Federation endemic for *O. felineus* infection [4, 5, 14–16].

Currently, there is more and more epidemiological evidence in favor of carcinogenicity of *O. felineus* trematode, which plays a role in the development of periductal fibrosis and CCA [4, 5, 11, 16].

CCA is one of the most unfavorable malignant neoplasms in terms of prognosis due to its long asymptomatic course, resistance to therapy, and

high mortality [8]. In connection with the above, it is of interest to search for modern methods of preclinical diagnostics of periductal liver fibrosis and CCA in the population of endemic regions.

The methods of ultrasonic diagnostics, as well as CT and MRI with contrast are used to diagnose CCA, which are reasonable to clarify the diagnosis, but cannot be used for screening due to the high cost, duration of the procedure, burden on the patient's body and possible contraindications. At the same time, the currently used diagnostic tests to assess the level of oncomarkers do not have sufficient sensitivity and specificity, but in conjunction with additional biochemical blood parameters, it is possible to assess the stage of hepatic fibrosis as a precancerous condition, which can be recommended for screening study.

Thus, the most sensitive and specific marker of CCA is cancer antigen CA 19-9: the increase of its content in serum above 100 U/mL can be used for CCA diagnosis with sensitivity, according to different data, from 72 to 97 % and specificity 84–86 % [21, 22]. Our study showed that the level of CA 19-9 in the serum of patients with CCA and opisthorchiasis was significantly higher compared to patients infected with *O. felineus* with periductal fibrosis and the control group ($p < 0.01$). No significant differences were obtained for this marker in Groups 2–4; however, the present study found that changes in the level of biochemical parameters (alkaline phosphatase, direct bilirubin, AST, glucose) as well as CA 19-9 oncomarker in combination are significant predictors of periductal liver fibrosis ($p < 0.05$).

Recently, the study of the diagnostic value of TGF- marker — extracellular matrix protein, which is a marker of the signaling pathway in CCA metastasis, has also become relevant [23]. The next most sensitive and specific marker is cytokeratin 19 fragments (CYFRA 21-1), which can distinguish between hepatocellular carcinoma and intrahepatic CCA [21, 24]. Nevertheless, reproducibility, sensitivity and specificity of more than 90 % in the detection of CCA can be achieved only when CYFRA 21-1 is used in combination with other markers and studies [25].

A study of the metabolomic response to *O. felineus* infection showed an association between serum lactate concentration and the severity of periductal fibrosis in the liver, an established predictor of CCA development [26–29]. In our study, serum lactate levels were significantly lower in patients with CCA than in Groups 2–4. According to published data, this may be a predictor of the development of multi-organ failure in patients with terminal diseases [30].

Conclusion

Thus, the first pilot data on the relationship between ultrasonometric signs of periductal liver fibrosis and increased biochemical parameters (alkaline phosphatase, direct bilirubin, aspartate aminotransferase, glucose), as well as increased CA 19-9 oncomarker were obtained, which can be used for early diagnosis of malignant transformation of periductal liver fibrosis with the formation of cholangiocarcinoma in *O. felineus*-infected population of endemic regions.

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