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Clinical and immunological features of opisthorchiasis

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Abstract

Aim. To assess the clinical and immunological features in patients with chronic opisthorchiasis, depending on the duration of the infection.

Methods. The first group consisted of 19 patients with the duration of the infection up to 1 year, the second group consisted of 21 patients with the duration of the infection between 1 and 5 years, the third group was formed of 23 patients with the duration of the disease more than 5 years, the control group — 20 healthy individuals. Immunological research was carried out at the Clinical Diagnostic Center. Statistical processing was performed using Microsoft Excel 2010 and Statistica 6.0 software. The statistical significance of differences was determined by using the Mann–Whitney test (U-test) at the level of significance of $p < 0.05$. The correlations were assessed by calculating Spearman's rank correlation coefficients.

Results Clinical features of chronic opisthorchiasis were revealed in the disease duration groups of up to 1 year, from 1 year to 5 years, more than 5 years: the subclinical course was most common in the group of up to 1 year; cholangiohepatitis prevailed in the group of between 1 to 5 years, allergic skin syndrome, cholangiocholecystitis and pancreatitis dominated in the group of more than 5 years. The immune response in chronic opisthorchiasis was characterized by: up to 1 year — lymphocytosis, increased levels of immunoglobulins M (IgM) and circulating immune complexes (CIC), a decrease in the number of T-lymphocytes ($CD3^+$), as well as an increase in bactericidal activity of leukocytes (BAL); between 1 and 5 years — monocytosis, increased levels of immunoglobulins M, immunoglobulins G and circulating immune complexes, a decrease in T-cytotoxic lymphocytes ($CD8^+$) and nitro blue tetrazolium (NBT test), as well as an increase in NK cells and phagocytic activity of monocytes, more than 5 years — eosinophilia.

Conclusion. Common features of rearrangement of the immune system in opisthorchiasis: inflammatory changes in the hemogram, activation of humoral immunity with parallel suppression of the cellular component of the immune system, and increased phagocytosis.

Keywords: clinical picture, immune status of chronic opisthorchiasis.

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Background. Chronization of the pathological process in opisthorchiasis is caused by long-term parasitism of helminths in the organs of the hepatobiliary system and the pancreas and toxic effects on the organs of the gastrointestinal tract, resulting in chronic cholecystitis, cholangitis, hepatitis, pancreatitis, gastritis, duodenitis, gastric ulcer, and duodenal ulcer [1–3].

Several authors associate the emergence of active hepatitis with immune inflammation, especially in cases of superinvasion and reinvasion [4]. Some patients develop pancreatitis as characterized by an undulating course with a frequent change in periods of exacerbations and remissions, with rare progressions. Opisthorchiasis is often accompanied by skin lesions, which are a consequence of the allergic parasite invasion reaction of the body [5]. Allergic skin syndrome in chronic opisthorchiasis can be manifested by pruritus, erythema, urticaria, and in severe cases, Quincke's edema, as well as peripheral blood eosinophilia [5, 6].

In the applied aspect, questions concerning the pathophysiological mechanisms of the immune system functioning under chronic invasion conditions are the most relevant [7]. Several studies showed that the count of T-helpers ($CD4^+$) is reduced in the active stage of the disease, and the count of T-cytotoxic cells ($CD8^+$) increased [8]. In addition, patients with chronic opisthorchiasis, who had a high intensity of invasion, exhibit significantly decreased counts of CD^{3+}, $CD8^+$, and $CD16^+$ cells in the peripheral blood with simultaneously increased

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$CD19^+$ B-lymphocytes compared to the group with a low invasion intensity [9].

Macrophages are involved in protecting the body from parasitic invasions [10, 11]. Moreover, they are activated in some cases, which contribute to the body's combat against invasion, they are suppressed in others, which determine the further development of the disease to a certain extent, and sometimes an unfavorable outcome [12].

Eosinophils play a special role in the implementation of antiparasitic immunity in opisthorchiasis invasion [13]. Eosinophilic granulocytes in helminthiases perform various functions, including numerous antigen-antibody complex phagocytosis, hypersensitivity modulation, and helminth killings with the participation of immunoglobulins (Ig) G [14].

Concurrently, the effect of opisthorchis metabolites on the immune response based on the invasion duration remains open, since this information is only experimental.

This study aimed to assess the clinical and immunological characteristics of patients with chronic opisthorchiasis based on the invasion duration.

Materials and methods. This open prospective study was conducted in Yekaterinburg in the infectious diseases department No. 3 of the City Clinical Hospital No. 40. Group 1 consisted of 19 patients (7 males and 12 females) with up to 1 year of invasion duration and aged 42.4 ± 1.73 years. Group 2 included 21 patients (10 males and 11 females) with 1–5 years invasion duration and aged 41.4 ± 1.24 years. Group 3 included 23 patients (13 males and 10 females) with >5 years the invasion duration and aged 45.8 ± 1.56 years.

Inclusion criteria:

1) males and females aged 18 to 65 years;

2) confirmed diagnosis of chronic opisthorchiasis by detecting *Opisthorchis felineus* eggs in feces and/or bile;

3) voluntary consent of patients to dehelminthization and laboratory testing;

4) voluntary consent of patients to participate in a clinical trial; and

5) residence in the Sverdlovsk region.

Exclusion criteria:

1) concomitant other parasitic pathology;

2) chronic hepatic injury not caused by opisthorchiasis (viral hepatitis, autoimmune hepatitis, and metabolic liver diseases);

3) pregnancy and breastfeeding; and

4) acute infectious disease, 3 months before the start of the study.

This study was approved by the regional ethical committee of the Ural State Medical University of the Ministry of Health of the Russian Federation, protocol No. 10 of 12/18/2015.

The diagnosis of chronic opisthorchiasis in all patients was established based on clinical, epidemiological, coproovoscopic, and bilioovoscopic data. Coproovoscopy was performed by Kato thick smear using cellophane and chemical sedimentation method. All 63 patients were found to have eggs of the Siberian fluke.

Immunological research was performed at the Clinical Diagnostic Center, with the written permission of patients. The general blood test parameters were recorded using a CobasMicros 60 (ABX) hematological analyzer. Immunophenotyping of lymphocytes was performed using monoclonal antibodies CD3-FITC/CD20-PE, CD3-FITC/CD4-PE, CD3-FITC/CD8-PE, and CD3-FITC/CD16+56-PE (IOTest) by flow cytofluorometry on a FAC Scan cytometer (Becton Dickinson). The amount of Ig of classes M, G, and A in blood serum was determined using the method of radial immunodiffusion in agar gel according to G. Mancini. The amount of circulating immune complexes (CIC) was determined by their precipitation in a 4% solution of PEG-6000 according to V. Haskova modified by Yu.A. Grinevich. Results were evaluated in extinction units using spectrophotometry on an SF-46 apparatus. The functioning of the nicotinamide-adenine dinucleotide phosphate-oxidase[1] system of neutrophils was assessed using the nitro blue tetrazolium test (NBT test).

A method developed in the laboratory of the Institute of Immunology of the Ministry of Health of the Russian Federation was used to assess the leukocyte intracellular killing (bactericidal activity), the phagocytosis completeness, and the neutrophil and monocyte absorptive activity.

An immunological study was performed before the anthelmintic therapy started. The main criteria to determine the invasion duration included the epidemiological history (time of consumption of carp fish) and the duration of clinical symptoms. All study participants ate carp fish. The control group, formed by the method of random sampling, consisted of 20 somatically healthy individuals who had no carp fish in their diet during their life and had negative fecal results for helminth eggs.

Statistical processing of the obtained data was performed using the electronic programs Microsoft Excel 2010 and Statistica 6.0. Methods of nonparametric analysis using the Mann–Whitney U-test were used to compare the obtained data. The statistical significance of the differences was recognized when the calculated value of the *U*-test was equal to or less than the critical value. The intensity of the correlation connection (*r*)

[1] NADP: nicotineamide-adenine dinucleotide phosphate.

Table 1. Epidemiological characteristics of the course of chronic opisthorchiasis in patients based on invasion duration

Diet	Invasion up to 1 year (*n* = 19)		Invasion from 1 to 5 years (*n* = 21)		Invasion over 5 years (*n* = 23)	
	n	%	n	%	n	%
Only carp fish	3	15.7	3	14.2	4	17.3
Seawater fish and carp fish (including sun-dried fish)	16	84.3	18	85.8	19	82.6
Fish from the water reservoirs of the Sverdlovsk region	16	84.2	19	90.4	17	73.9
Fish from water reservoirs of the Tyumen region and the Khanty-Mansiisk autonomous district	2	10.5	1	4.8	4	17.4
Fish from the Ob-Irtysh basin	1	5.3	1	4.8	2	8.7

Table 2. Clinical classification of opisthorchiasis (according to N.N. Ozeretskovskaya)

Phases of the disease	Clinical syndrome	Organ lesions (clinical course)
Acute	Subclinical. Primary: general allergic manifestations, hepatocholangitis, gastroenteric, and paratyphoid. Rare: angioedema of the larynx, Lyell's syndrome, delirium, etc.	Cholangiocholecystitis, hepatitis, pancreatitis, gastritis (catarrhal, erosive), enterocolitis, gastric and duodenal ulcers, asthma-like bronchitis, pneumonia, cerebral edema, etc.
Acute superinvasion	Similar to acute-phase syndromes	Similar to the acute phase
Chronic	Subclinical. Dyspeptic, asthenoneurotic, S.P. Botkin cholecystocoronary, M.P. Konchalovsky pancreatic coronary, pulmonary, etc.	Cholangiocholecystitis, cholangiohepatitis, pancreatitis, gastroduodenitis, allergic skin, asthma-like bronchitis, gastric and duodenal ulcers, stenosing papillitis, etc.
Superinvasion in the chronic phase	Similar to acute-phase syndromes	Similar to acute-phase lesions
Reinvasion	Similar to acute-phase syndromes	Similar to acute-phase lesions
Residual period of the acute phase	Reverse development of the disease acute phase	Reverse development of the acute-phase organ lesions
Residual period of the chronic phase	Compensation or stabilization of the chronic phase	Clinical compensation or stabilization of organ lesions in the chronic phase

was assessed using Spearman's rank correlation coefficient.

Results and discussion. Taking into account the data of the epidemiological history of our patients, identifying the following patterns was possible (Table 1). The diet included only carp fish (roach, carp, asp, rudd, etc.) to an equal extent in patients with up to 1 (15.7%) year, 1–5 (14.2%) years, and >5 (17.3%) years of invasion. Sea and carp fish (including air-dried fish) were used by 84.3% of patients with up to 1 year, by 85.8% with 1–5 years, and by 82.6% with >5 years of invasion duration. The diet included mainly fish caught from the reservoirs of the Sverdlovsk region was registered in 84.2% of patients with up to 1 year, in 90.4% with 1–5 years, and 73.2% with >5 years of invasion duration. Fish brought from the water bodies of the Tyumen region and the Khanty-Mansiisk autonomous district (17.4%) and the Ob-Irtysh basin (8.7% of cases) were consumed most often by patients with >5 years of invasion duration.

The classification of N.N. Ozeretskovskaya et al. (1985) was used to make a diagnosis (Table 2), according to which, the following syndromes were distinguished in the clinical presentation of our patients.

The subclinical course prevailed (15.7%) in up to 1 year of invasion duration, in which distinctive aspect includes asthenia, decreased performance, and sleep disturbance. The subclinical course with the duration of the disease from 1 to 5 years was revealed in 9.5% of patients, and in 8.7% of cases with >5 years of invasion, which was characterized by rapid fatigue and irritability. This variant is because, in the foci of opisthorchiasis invasion in the indigenous and local population, adaptive relationships could develop in the host's defense system and in the parasite's defense and aggression systems, which provide the possibility of their

long-term coexistence under conditions of chronic invasion [3, 15].

From 1 to 5 years of invasion, cholangiohepatitis dominated (57.1%), characterized by pain in the right hypochondrium (81.8%), spicy and fried food intolerance (41.6%), nausea (33.3%), skin pruritus (16.6%), and hepatomegaly (25.0%). Less often, this syndrome developed with up to 1 year of invasion (31.5%), with the major symptom of heaviness in the right hypochondrium (83.3%). A distinctive aspect of cholangiohepatitis after invasion for >5 years (39.1%) was pain and heaviness in the right hypochondrium (77.7%) and decreased appetite (55.5%).

Most probably, the ascending route of opisthorchis and its metabolites was significant in the pathogenesis of this syndrome with a mechanical and reflex effect, as well as a secondary infectious factor, which was implemented by a descending (hematogenous) route that influences the intrahepatic bile ducts and the liver [4, 16].

Allergic skin syndrome prevailed in 47.8% of cases with >5 years of invasion, in 26.3% with up to 1 year, and in 28.5% with 1–5 years of invasion. It was characterized by a wavy course of recurrent urticaria, the most significant symptoms of which were red or pale pink blisters that are mainly localized on the abdomen, back, upper and lower extremities, and in the decollete area. Elements of the eruption were up to 7–10 cm in diameter, which spread to large areas of the body. Pruritus was noted throughout the day, which often causes insomnia, as well as a periodic rise in body temperature to subfebrile indices.

Allergic restructuring of the body in patients arose, apparently, as a result of the toxic and sensitizing effects of metabolic products and the decay of opisthorchis, as well as, possibly, autosensitization by the decay products of their tissues (epithelial cells lining the bile ducts, which undergo necrosis when they are traumatized by helminths) [3, 5].

Cholangiocholecystitis was also most common after >5 years of invasion that accounted for 65.2%, whereas 42.1% in up to 1 year and 38.1% in 1–5 years of invasion duration. The main symptoms of cholangiocholecystitis include the following:

– in >5 years of invasion duration, pressing pains in the right hypochondrium that radiates to the left clavicle (53.3%), belching (33.3%), epigastric burning (26.6%), and a positive Ortner's symptom (13.3%);

– in up to 1 year of invasion duration, pain in the right hypochondrium, radiating to the right half of the chest (37.5%), nausea (62.5%), and low-grade fever (25.0%);

– in 1–5 years invasion from, stabbing pain in the right hypochondrium with irradiation to the right clavicle (37.5%), fatty foods intolerance (75.0%), and a positive Kehr's symptom (12.5%).

The mechanical and damaging action of parasites on the epithelial cells of the hepatobiliary system is of great importance in the pathogenesis of cholangiocholecystitis. Their bothria and spines capture the duct wall sections, accumulate in them, create an obstacle for the normal bile outflow, and subsequently contribute to the creation of favorable conditions for the secondary bacterial infection [2, 17, 18].

The pathogenesis of chronic pancreatitis includes the entrance of *Op. felines* and its waste products into the pancreatic ducts from the duodenum through the hepatopancreatic ampulla with a further increased intraductal pressure and obstruction of the outflow of juice and secretions, as well as activation of the secondary microflora and its enzymes [3, 15, 17, 18]. Distinctive clinical signs of chronic pancreatitis (30.4%) as a key variant with invasion for >5 years include nagging pains in the umbilical region (71.4%), radiating to the right scapula (40.0%), abdominal distention (85.7%), and bitter taste in the mouth (57.1%). In 1–5 years of invasion, pancreatitis developed in 19.1% of patients and was characterized by stabbing pains in the left hypochondrium (25.0%), abdominal distention (75.0%), and flatulence (50.0%). In >1 year of invasion duration, the pancreatic lesion was rare (10.5%) and the clinical manifestations reduced to girdle pain in the abdomen, loose stools, and decreased appetite (Table 3). Our patients, with >5 years of invasion duration had the most prolonged relapses of cholangiocholecystitis (3.08 ± 1.02 months), as well as allergic skin course (2.65 ± 0.78 months).

A study of the immune status was performed to identify various changes in immunological reactivity in our patients of three groups.

Our study revealed that the development of a chronic immune-inflammatory process in opisthorchiasis based on the disease duration had certain characteristics (Table 4), namely, increased level of lymphocytes, cells that form the first line of defense against helminths, was recorded with invasion up to 1 year (2.74 ± 0.33; $p = 0.021$) and >5 years (2.66 ± 0.54; $p = 0.032$) compared with the control group (2.41 ± 0.18; $p = 0.042$).

Group 2 had lymphocyte indices within the reference values (1.81 ± 0.32; $p = 0.081$). Increased count of monocytes (0.89 ± 0.06; $p = 0.047$), which are cells responsible for nonspecific defense of the body, as well as those that play a significant role in the process of inflammation and antiparasitic protection, was registered compared with the indicators of healthy volunteers (0.58 ± 0.04; $p = 0.038$). Monocyte indices in group 1 (0.76 ± 0.05;

Table 3. Clinical presentation of chronic opisthorchiasis based on invasion duration

The clinical course of chronic opisthorchiasis	Invasion up to 1 year (n = 19)		Invasion from 1 to 5 years (n = 21)		invasion more than 5 years (n = 23)	
	n	%	n	%	n	%
Subclinical course	3	15.7	2	9.5	2	8.7
Cholangiohepatitis	6	31.5	12	57.1	9	39.1
Allergic skin syndrome	5	26.3	6	28.5	11	47.8
Cholangiocholecystitis	8	42.1	8	38.1	15	65.2
Pancreatitis	2	10.5	4	19.1	7	30.4

Table 4. Immunological parameters in patients with chronic opisthorchiasis, depending on the invasion duration

Parameter	Group of patients, M ± m			Control group (n = 20)
	Group 1: invasion up to 1 year (n = 19)	Group 2: invasion from 1 to 5 years (n = 21)	Group 3: invasion more than 5 years (n = 23)	
Lymphocytes, $\times 10^9$/L	2.74±0.33*	1.81±0.32	2.66±0.54*	2.41±0.18
Monocytes, $\times 10^9$/L	0.76±0.05	0.89±0.06*	0.64±0.05	0.58±0.04
Granulocytes, $\times 10^9$/L	3.26±1.22	4.12±1.65	2.74±0.37	4.21±0.98
Eosinophils, $\times 10^9$/L	0.16±0.02	0.18±0.03	0.34±0.03*/***	0.29±0.02
Erythrocyte sedimentation rate, mm/h	12.6±0.98	14.1±1.23	11.7±1.12	12.5±1.71
Immunoglobulins M, g/l	1.74±0.32*	1.66±0.43*	1.68±0.48*	1.24±0.29
Immunoglobulins G, g/l	13.6±1.38	18.4±1.76*	20.6±1.95*	12.9±1.09
Circulating immune complexes, units	106.2±2.61*/***	78.4±1.96*	58.9±1.88	54.2±3.11
$CD19^+$	0.17±0.02	0.26±0.03	0.34±0.04	0.27±0.02
$CD3^+$	0.97±0.04*	1.36±0.05	0.89±0.05*	1.24±0.13
$CD4^+$	0.59±0.03	0.58±0.02	0.56±0.02	0.76±0.19
$CD8^+$	0.56±0.02	0.45±0.03*	0.76±0.03	0.59±0.11
NK cells	0.26±0.01	0.49±0.03*/**	0.36±0.02*	0.28±0.04
Stimulated test with nitroblue tetrazolium, %	26.1±1.39	12.7±1.01*/**	14.4±1.48*	28.1±2.78
Bactericidal activity of leukocytes, %	63.2±2.84*/**	34.9±2.32	42.1±2.66	46.7±3.34
Absorption activity of monocytes, %	69.1±2.04	82.4±2.81*	79.4±2.11*	57.4±3.97
Absorption activity of neutrophils, %	89.4±2.96	87.2±3.03	92.4±3.07	83.1±4.59

Note: the level of significance of differences is $p < 0.05$: *when compared with the control group; **when comparing the invasion duration up to 1 year with the invasion duration from 1 to 5 years; ***when comparing the invasion duration up to 1 year and the invasion duration more than 5 years (nonparametric Mann–Whitney U-test).

$p = 0.074$) and group 3 (0.64 ± 0.05; $p = 0.092$) were within the normal range.

The highest count of eosinophils, which most likely attached to helminths, locally released the contents of the granules and damaged the shell of the parasite, and were also involved in inflammatory reactions [13, 14, 19], was detected in case of >5 years of invasion (0.34 ± 0.03; $p = 0.046$) compared with the level in group 1 (0.16 ± 0.02; $p = 0.036$) and of healthy people (0.29 ± 0.02; $p = 0.084$).

Humoral link changes in increased level of Ig M was detected in all groups, namely with invasion up to 1 year (1.74 ± 0.32; $p = 0.033$), 1–5 years (1.66 ± 0.43; $p = 0.038$), and >5 years (1.68 ± 0.48; $p = 0.043$) compared with the control group (1.24 ± 0.29; $p = 0.044$). The highest concentration of CIC, which primarily reflect the anthelmintic load, was detected with up to 1 year of invasion (106.2 ±

2.61; $p = 0.041$) compared with the group 3 (58.9 ± 1.88; $p = 0.065$) and the control group (54.2 ± 3.11; $p = 0.088$). Increased IgG synthesis, which indicated a long-term active chronic allergic inflammation and its participation in the secondary immune response, was recorded with 1–5 years (18.4 ± 1.76; $p = 0.029$) and >5 years of invasion (20.6 ± 1.95; $p = 0.024$) compared with the control group (12.9 ± 1.09; $p = 0.025$).

In groups 1 (0.97 ± 0.04; $p = 0.027$) and 3 (0.89 ± 0.05; $p = 0.039$), decreased count of CD^{3+} lymphocytes was revealed in the cellular link of immunity compared with the values of healthy volunteers (1.24 ± 0.13; $p = 0.045$), which can be considered as an immune response to the invasion of a pathogen into the internal environment. This characterized the onset of the formation of a protective response [8, 20]. Meanwhile, with 1–5 years of invasion, an imbalance in the cellular link of immunity was recorded with decreased count of $CD8^+$ lymphocytes (0.45 ± 0.03; $p = 0.042$) compared with the control group (0.59 ± 0.11; $p = 0.076$), as well as the highest indices of natural killer (NK) cells (0.49 ± 0.03; $p = 0.041$) compared with group 1 (0.26 ± 0.01; $p = 0.034$) and healthy people (0.28 ± 0.04; $p = 0.055$), indicating a compensatory-adaptive response of T cells under conditions of prolonged antigenic stimulation of *Opisthorchis felineus* [8, 21, 22].

Among the nonspecific defense mechanisms, the highest increase in the bactericidal activity of leukocytes (63.2 ± 2.84; $p = 0.021$) was registered in cases of up to 1 year of invasion duration, compared with group 2 (34.9 ± 2.32; $p = 0.041$) and the control group (46.7 ± 3.34; $p = 0.062$), leading to a complete potential with the ability of the phagocytic link of immunity to becoming one of the main participants in the response immuno-inflammatory reactions occurring in the tissues of the macroorganism [10, 11, 23].

The phagocytic link of immunity in group 2 (12.7 ± 1.01; $p = 0.033$) compared with the values of group 1 (26.1 ± 1.39; $p = 0.038$) and healthy people (28.1 ± 2.78; $p = 0.054$) revealed the lowest indices of the NBT test, which probably was associated with absorptive function weakening of phagocytes that was replenished by the absorptive activity of monocytes (82.4 ± 2.81; $p = 0.047$) compared with the indices of the control group (57.4 ± 3.97; $p = 0.065$) [20, 23]. In turn, in >5 years of invasion, in presence of the decrease in the NBT test (14.4 ± 1.48; $p = 0.045$) compared with the control group and activation of the absorption activity of monocytes (79.4 ± 2.11; $p = 0.048$) compared with healthy volunteers, an increase in the count of NK cells (0.36 ± 0.02; $p = 0.047$) was revealed compared with healthy people, which could indicate the mutual adaptation of the parasite and the T-cell link of immunity for a long period [8, 20, 22].

Subsequently, the relationship between the duration of opisthorchiasis invasion and immunological parameters was evaluated (Table 5). A friendly reaction was established between the invasion duration and the number of CIC, including a strong correlation in case of up to 1 year invasion ($r = +0.811$; $p = 0.022$), whereas a weak direct correlation in 1–5 years ($r = +0.284$; $p = 0.036$) and >5 years of invasion ($r = +0.161$; $p = 0.042$). Increased concentration of immune complexes promoted humoral immune protection against helminth antigens, which they bound, neutralized, and subsequently destructed/removed from the body [24, 25] (Fig. 1).

In addition, an antagonism of up to 1 year ($r = -0.028$; $p = 0.042$) and >5 years of invasion duration ($r = -0.022$; $p = 0.032$), as well as a strong positive relationship of duration from 1 to 5 years ($r = +0.781$; $p = 0.034$) with CD^{3+} lymphocytes. The increased CD^{3+} lymphocyte counts, were most likely indicative of immune system hyperactivity, and, indicated a sluggish course of the chronic inflammatory process against the polymorphism of clinical manifestations [8, 26] (Fig. 2).

Regardless of the invasion duration, synergism with IgG was recorded for cases up to 1 year ($r = +0.024$; $p = 0.046$), 1–5 years ($r = +0.845$; $p = 0.041$), and >5 years of invasion duration ($r = +0.281$; $p = 0.044$). The waste products of *Opisthorchis felineus*, apparently resulted in immunosuppression in the course of chronicity and became a powerful trigger for the synthesis of IgG, which was involved in the secondary immune response [25, 27] (Fig. 3).

A negative relationship of average strength was revealed between the invasion duration up to 1 year ($r = -0.34$; $p = 0.041$), as opposed to a direct dependence in 1–5 years ($r = +0.038$; $p = 0.026$), as well as for >5 years (+0.797; $p = 0.045$), and T-helpers, which indicates that $CD4^+$ actively participated in the formation of an adaptive immune response, perceived the metabolic products of the cat liver fluke as an irritant and subsequently, possibly, transmitted signals to B-cells and T-effectors [22, 28] (Fig. 4).

A weak negative correlation was noted between the 1–5 years of invasion duration ($r = -0.027$; $p = 0.034$) and T-cytotoxic lymphocytes. Increased production of $CD8^+$ lymphocytes in patients with up to 1 year ($r = +0.213$; $p = 0.037$) and >5 years ($r = +0.835$; $p = 0.035$) of invasion is possibly due to a cellular response with longer antigenic stimulation [8, 25, 29] (Fig. 5).

Consequently, in the chronic phase of opisthorchiasis invasion, the intensity of the phagocytic,

Table 5. Correlation between the invasion duration and immunological parameters in patients with chronic opisthorchiasis ($p < 0.05$)

Clinical group	Indicator				
	Circulating immune complexes	$CD3^+$	Immunoglobulins G	$CD4^+$	$CD8^+$
Chronic opisthorchiasis with up to 1 year of invasion duration ($n = 19$)	+0.811	–0.028	+0.024	–0.34	+0.213
Chronic opisthorchiasis with 1–5 years of invasion duration ($n = 21$)	+0.284	+0.781	+0.845	+0.038	–0.227
Chronic opisthorchiasis with >5 years of invasion duration ($n = 23$)	+0.161	–0.022	+0.281	+0.797	+0.835

Note: $r = 0$–0.3 indicated weak correlation; $r = 0.3$–0.7 indicated moderate correlation; $r = 0.7$–1 indicated strong correlation; in the case of a "+" result, the relationship was direct, whereas a "-" result indicated an inverse relationship.

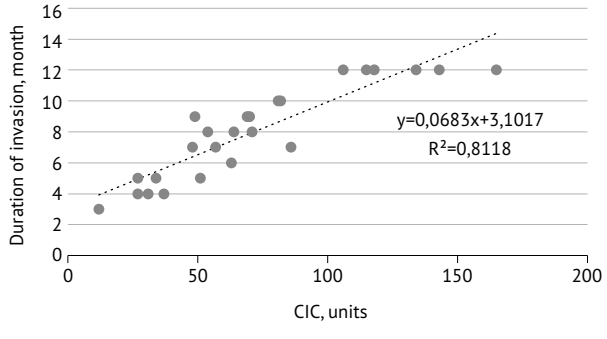

Fig. 1. Correlation between up to 1 year of invasion duration and the level of circulating immune complexes (CIC).

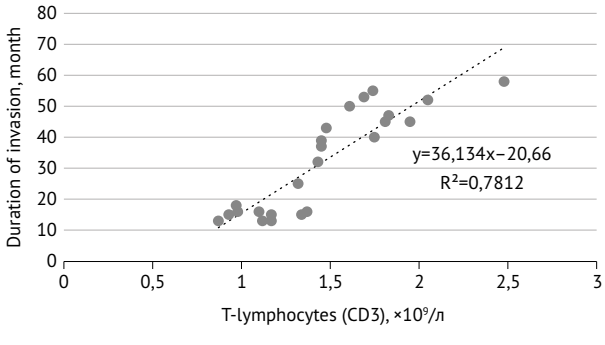

Fig. 2. Correlation between 1–5 years of invasion duration and the level of CD3+ lymphocytes.

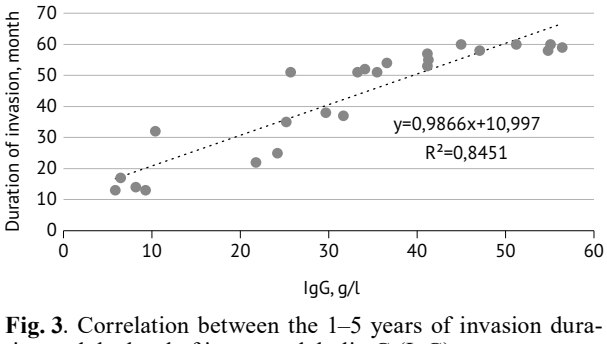

Fig. 3. Correlation between the 1–5 years of invasion duration and the level of immunoglobulin G (IgG).

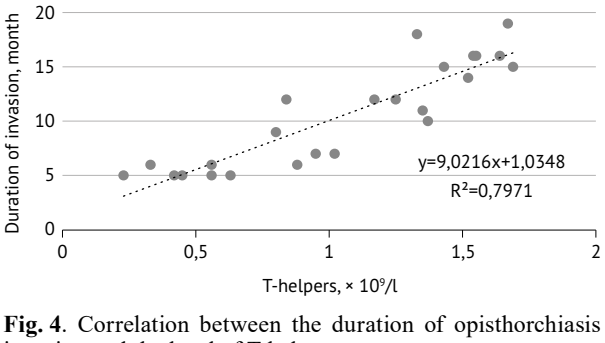

Fig. 4. Correlation between the duration of opisthorchiasis invasion and the level of T-helpers.

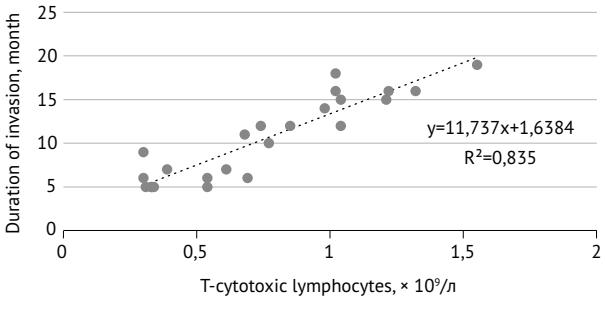

Fig. 5. Correlation between the duration of opisthorchiasis and the level of cytotoxic lymphocytes.

cellular, and humoral links of immunity in patients depended on the disease duration and the eosinophilic reaction.

CONCLUSIONS

1. In patients with chronic opisthorchiasis, the classical clinical presentation prevailed with the course variants of cholangiocholecystitis, cholangiohepatitis, and pancreatitis. The invasion that lasts for >5 years is characterized by the frequent and prolonged relapse of the allergic skin syndrome (2.65 ± 0.78 months).

2. The immune response in chronic opisthorchiasis was characterized by (1) lymphocytosis, increased IgM and CIC levels and decreased CD^{3+} lymphocyte counts, as well as increased bactericidal activity of leukocytes, in case of up to 1 year of invasion duration; (2) monocytosis, increased IgM, IgG, and CIC counts, decreased $CD8^{+}$ lymphocytes levels, and test results with NBT, as well as increases NK cells counts and activation of the absorption activity of monocytes, in case of 1–5 years; (3) eosinophilia in >5 years of invasion duration.

3. General characteristics of immunological restructuring in opisthorchiasis invasion include inflammatory changes in the hemogram and activation of humoral immunity with a parallel cellular link suppression, as well as strengthening of nonspecific defense mechanisms.

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