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Case Report: Two Cases of Cholangiocarcinoma in Patients with *Opisthorchis felineus* Infection in Western Siberia, Russian Federation

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Abstract. Cholangiocarcinoma (CCA) is a cancer with high mortality owing to its aggressiveness and resistance to therapy. The liver flukes of the Opisthorchiidae family have been recognized as risk factors of CCA. *Opisthorchis felineus* infection occurs in Western Siberia, the biggest endemic area in the Russian Federation, and is associated with chronic inflammation of the bile ducts, which may be linked to severe hepatobiliary morbidity. We report two cases of confirmed CCA who had a chronic *O. felineus* infection. Both cases presented unspecific symptoms at the onset of the disease, a stage when severe pathological changes already had occurred. Both patients were living in endemic areas but did not receive any antihelminthic treatment. This report underlines the need for assessment of *O. felineus* infection as a causative factor of CCA. The results will provide further arguments for control of *O. felineus* in the Russian Federation.

INTRODUCTION

Cholangiocarcinoma (CCA) is a group of cancers with pathologic features of intra- or extrahepatic biliary tract differentiation.[1] Cholangiocarcinoma is a rare form of cancer with highest incidence rates in Thailand, China, and South Korea.[2–4] Cholangiocarcinoma is a highly fatal tumor, clinically silent in most of the cases and resistant to existing therapies. Genetic background associated with primary sclerosing cholangitis, hepatitis C and B, duct cysts, bile stones, obesity, inflammatory bowel disease, tobacco smoking, poisons, and liver fluke infection are the most relevant risk factors for CCA.[5–14]

In Russia, the incidence of liver and bile duct cancer is around 4.8 cases per 100,000 population per year; the highest incidence has been reported in regions of Western Siberia and the Sakha Republic.[15]

We report two cases of confirmed CCA in patients from Western Siberia that suffered from chronic *Opisthorchis felineus* (*O. felineus*) infection.

CASE DESCRIPTION

Case 1. We describe the case of a 67-year-old man who has lived in Tomsk Oblast (Western Siberia) all his life. He practiced fishing in the rivers for nutritional purposes and was eating fried and undercooked (salted, smoked, or dried) *Cyprinidae* fish. He never used separate kitchen utensils for fish preparation. In 1980, he was diagnosed with *O. felineus* infection and treated with chloxylum (the antihelminthic treatment used before the official registration of praziquantel). After this diagnosis, he was not reexamined or re-treated for opisthorchiasis. At the end of 2015, he was admitted to the hospital with high fever which lasted 1 month. Antibacterial treatment was initiated, which decreased the patient's temperature. From March 2016 onward, he had fevers of up to 39°C three to four times per month and he suffered from weakness and weight loss (> 10 kg). In June 2016, jaundice appeared leading to clinical examination in the District Hospital of Tomsk Oblast. The erythrocyte sedimentation rate (ESR) was accelerated and biochemical analysis showed high levels of bilirubin and serum proteins (Table 1). Stool examinations were positive for *O. felineus* eggs, whereas abdominal ultrasonography revealed diffuse changes in the liver and pancreas, signs of chronic cholecystitis, and enlarged liver. Magnetic resonance imaging showed chronic cholecystitis, pancreatitis, enlarged liver, and enlarged biliary ducts (Figure 1). The common bile duct was enlarged to 1.0 cm in diameter in the proximal and middle parts.

Histology: A conventional small bile duct type of intrahepatic CCA was identified with a moderately differentiated tubular adenocarcinoma with desmoplastic reaction. A small ductular carcinoma grew in the desmoplastic stroma (Figure 2).

The diagnosis was IV stage, T4NXM1 (T4—tumor with periductal invasion, NX—regional lymph node cannot be assessed, M1—distant metastasis) intrahepatic periductal CCA (moderately differentiated tubular adenocarcinoma), and chronic *O. felineus* infection.

The patient underwent cholecystectomy, section of the confluence and the right hepatic duct, mechanical intestinal anastomosis, and hepatojeunostomy by three through-liver drainages. Intraoperatively, an enlarged liver was detected and purulent discharge was obtained from the bile ducts. The tumor was found in the hepatic portal vein area.

Microbial analysis of the bile duct contents revealed profuse growth of *Klebsiella* spp. The patient remained in a severe condition during the postoperative period and died from progressive liver failure 6 days after the operation in August 2016.

Case 2. A 66-year-old man from Tomsk Oblast is reported, who regularly ate raw or insufficiently cooked *Cyprinidae* fish since childhood. He had been ill since April 2017 when pain in the right hypogastrium initially appeared. He was admitted to the hospital in May 2017 with abdominal pain, jaundice, and weakness. Blood tests showed anemia and increased levels of ESR and increased alkaline phosphatase. Ultrasonography revealed an enlarged liver, diffuse changes in liver and

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TABLE 1
Results of laboratory and ultrasonographical examinations

Data	Case 1	Case 2
Laboratory examination		
Hemoglobin (110–150 g/L)	136	109
Leukocytes (4,000–9,000/L)	8.5	9.2
Neutrophils (50–65%)	70	67
Lymphocytes (20–40%)	22	18
Monocytes (2–9%)	8	2
Eosinophils (1–4%)	0	13
Erythrocyte sedimentation rate, mm/hour	46	51
Total serum protein (6.0–8.5 g/dL)	63	70
Glucose (3.5–5.5 mmol/L)	5.2	5.3
Total bilirubin (3–20 μmol/L)	107.4	53.5
Conjugated bilirubin (0–5 μmol/L)	90.1	37.7
Alanine aminotransferase (0–40 U/L)	81	25
Aspartate aminotransferase (0–40 U/L)	97	27
Alkaline phosphatase (0–250 U/L)	1,188	314
Confirmation of *Opisthorchis felineus* infection	Stool microscopy	Autopsy (adult worms in bile ducts) and histology
Microscopy of stool (*O. felineus*, EPG stool)	*O. felineus* eggs, EPG stool 1: 2,148; stool 2: 3,048	Not performed
Abdominal ultrasonography		
Left liver lobe height, cm	166	80
Gallbladder wall thickened	Yes	Yes
Gallbladder wall irregular or with a halo	Yes	Yes
Gallbladder sludge (floating echogenic material)	Yes	Yes
Bile duct dilated	Yes	Yes
Longest measurement of gallbladder length (pre-fatty meal), cm	105	90
Liver parenchyma	Mildly increased periportal echoes	Mildly increased
Liver mass (suspected cholangiocarcinoma)	No	No
Bile ducts	Wall irregular	Wall irregular
Other comments	Enlarged liver, diffuse changes in liver and pancreas, and signs of chronic cholecystitis and cholechoectasis	Enlarged liver, diffuse changes in liver, and signs of chronic cholecystitis and cholechoectasis

EPG = eggs per gram.

pancreas, signs of chronic cholecystitis, and enlargement of the common bile duct (Table 1).

Magnetic resonance imaging showed a mass-like enlargement in the right lobe of the liver, involvement of biliary cysts, and enlargement of regional lymph nodes (Figure 3). The liver had coarse contours and density was diffusely increased. Enlarged size and deformation of the S1 segment due to the presence of mass lesions with unclear contours expanding along the lower contour S5 was observed. Peripheral bile ducts were enlarged up to 6 mm. The common bile duct was not visualized and determined in the pancreatic part only, with a diameter of 5 mm. The mass lesion had a dimension of 87 × 46 × 47 mm. The regional lymph nodes were enlarged.

In May 2017, the patient underwent an endoscopic retrograde cholangiopancreatography (ERCP), endoscopic

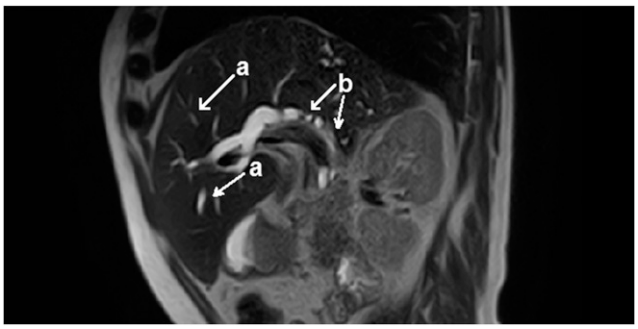

FIGURE 1. Case 1. Magnetic resonance imaging: signs of cholangiocarcinoma (axial view). (**A**) Enlarged intrahepatic bile ducts; (**B**) the common bile duct is not distinctly differentiated within 1.0 cm; its diameter is enlarged up to 1.0 cm in the proximal and middle part.

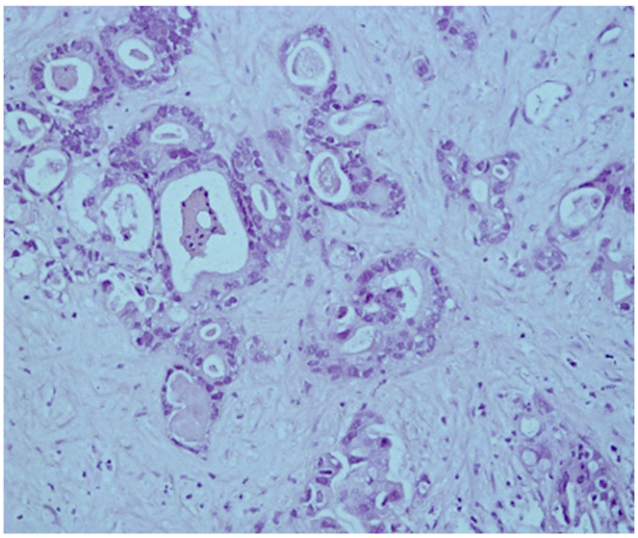

FIGURE 2. Case 1. Histology of liver parenchyma: a moderately differentiated tubular adenocarcinoma with desmoplastic reaction is found. A small ductular carcinoma grows in desmoplastic stroma. Hematoxylin and eosin stain (original magnification, ×40). This figure appears in color at www.ajtmh.org.

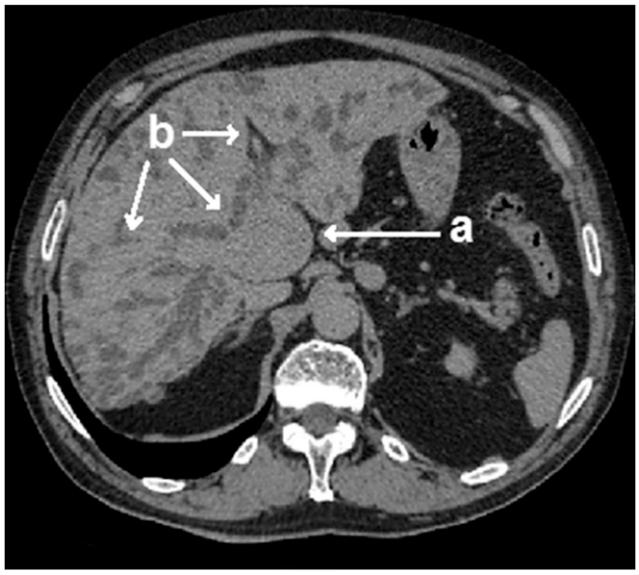

FIGURE 3. Case 2. Magnetic resonance imaging: signs of cholangiocarcinoma (sagittal view). Tumor in the right lobe of liver with bile hypertension, involving biliary cyst and defeat of regional lymphatic nodes. (**A**) Mass-like enlargement with unclear contours; (**B**) enlarged intrahepatic bile ducts.

papilla sphincterotomy, biopsy of bile ducts, and a nasobiliary drain.

Histology suggested an intrahepatic CCA. Epithelial cells were cuboidal with eosinophilic cytoplasm and round central nuclei, whereas the tumor cells were heterogeneous and resembled bile duct cells. There were signs of chronic cholangitis (Figure 4).

The patient underwent surgery (ERCP, bile duct bougienage, and bile duct bilateral stenting) in June 2017.

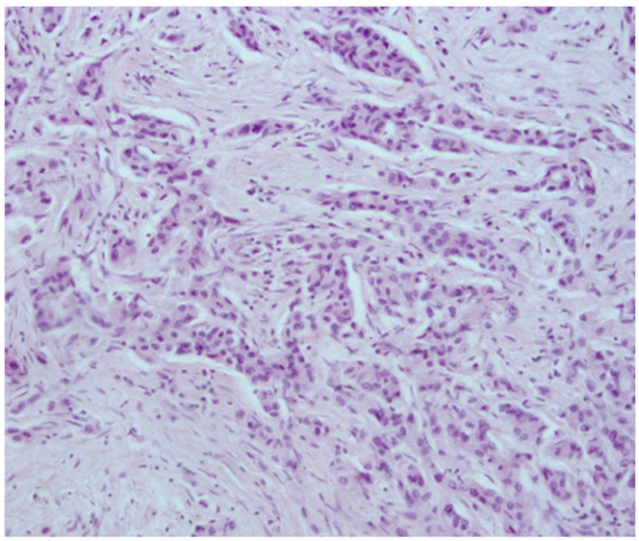

FIGURE 4. Case 2. Histology of liver parenchyma: bile ductular type of intrahepatic cholangiocarcinoma. Epithelial cells are cuboidal with eosinophilic cytoplasm and round central nuclei; tumor cells are heterogeneous and resemble bile duct cells. Poorly differentiated carcinoma grows in fibrous stroma and has cord-like growth. Hematoxylin and eosin stain (original magnification, ×20). This figure appears in color at www.ajtmh.org.

Two months later, he was admitted to the emergency department with abdominal pain, weakness, vomiting bile, fever (38.5°C), low blood pressure (40/0), and signs of ascites. He died of multiple organ failure 2 hours after admission to the clinic in August 2017.

The diagnosis was an intrahepatic mass-forming CCA (moderately differentiated ductular adenocarcinoma) with invasion of the walls of the portal vein and the hepatic artery and total infiltration of the gallbladder wall. A mass was found in the lower lobe of right lung: IV stage T4N1M1 (T4—tumor with periductal invasion, N1—regional lymph node metastasis, M1—distant metastasis). In addition, *O. felineus* infection was diagnosed.

The patient suffered from a series of complications: right-side serofibrinous pleuritis, acute gastric ulcers, and multiple necrosis in the liver. A general seropyoperitonitis led to a septic shock; bacterial analysis confirmed the growth of *Escherichia coli*. Multiple organ failure was the cause of death.

Autopsy showed enlarged liver (weight 1,580 grams) with "flabby" consistency; the parenchyma was light brown with multiple necrotic zones. The walls of the intrahepatic bile ducts were thickened and contained "muddy" bile. In the liver, the neoplasm was seen as a knot of dense consistency, 6 × 3 cm. Multiple areas of the tumor tissue were necrotic. The tumor involved the wall of the common hepatic duct and the wall of the cystic duct. The tumor compressed the portal vein and the hepatic artery. Multiple *O. felineus* adult worms were found in the bile ducts (Figure 5).

DISCUSSION

We presented two cases of CCA in patients originating from opisthorchiasis-endemic areas of Western Siberia and with chronic *O. felineus* infection. Both cases presented with unspecific symptoms at the onset of the disease at a stage when severe pathological changes had already occurred. The most common initial symptoms were fever, weakness, loss of appetite, weight loss, jaundice, and abdominal pain. Both

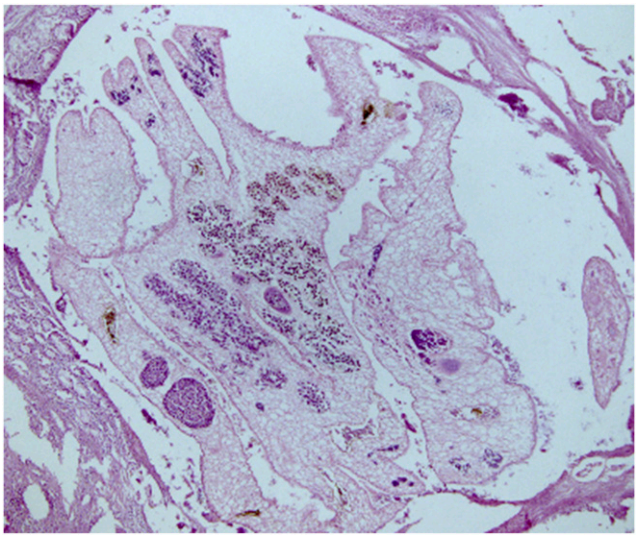

FIGURE 5. Case 2. Histology of liver parenchyma: *Opisthorchis felineus* adult worms in intrahepatic bile duct. Hematoxylin and eosin stain (original magnification, Plan ×5). This figure appears in color at www.ajtmh.org.

patients practiced fishing and were eating undercooked (raw) *Cyprinoid* fish for many years.[16] They were infected by *O. felineus* and did not receive any praziquantel treatment. Both cases developed CCA in their sixties and died after a relatively short illness from severe complications.

One of the most important documented risk factors of CCA is the long-term exposure to Asian fluke infections (*Opisthorchis viverrini* and *Clonorchis sinensis*).[14] The association between chronic *O. viverrini* infection and CCA development is well documented.[17–19] Northern Thailand has areas of highest prevalence of *O. viverrini* infection, and there is a relatively high proportion of CCA among the liver cancers diagnosed.[19] The carcinogenic potential of liver flukes due to combination of mechanical damage, parasite secretome, and immunopathology that may cause inflammation, periductal fibrosis, and proliferative responses predisposes individuals to CCA after long-standing *O. viverrini* infection.[17–20]

However, although suggestive for a causal relationship between chronic *O. felineus* infection and the development of CCA, evidence has not been proven yet. Experimental studies, autopsy results, and surgical reports, for instance, have demonstrated the carcinogenic potential of *O. felineus*. They show that long-term infestation causes periductal inflammation, periductal fibrosis, and proliferative responses which may represent predisposing lesions to malignancy.[21,22] *Opisthorchis felineus* infection in combination with other hepatotropic cancerogenic factors such as hepatitis C might significantly increase the risk for developing bile duct cancer.[23] Recently, the analysis of official medical statistics showed the importance of opisthorchiasis in endemic areas of the Russian Federation and presented data associating *O. felineus* infection and liver/bile duct cancers.[15]

CONCLUSION

This report underlines the need for a solid assessment of *O. felineus* infection as a causative factor for CCA. The results will provide further arguments for *O. felineus* control in the Russian Federation.

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Ethical approval: Study procedures followed the ethical standards of the Helsinki Declaration of the World Medical Association. Ethical Approval was obtained from the Ethical Committee of Siberian State Medical University (N4384, November 30, 2015). Patient's written informed consent was obtained. All information was anonymized.

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