

Diagnosis of Indolent *Clonorchis sinensis* and *Opisthorchis viverrini* Infections as Risk Factors for Cholangiocarcinoma: An Unmet Medical Need

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Background: Cholangiocarcinoma is a highly aggressive cancer of the biliary tract epithelium. This form of cancer is prevalent in Asia, and recent reports show that its incidence is relatively rare but increasing in the United States. Although risk factors for cholangiocarcinoma have yet to be elucidated, a growing body of literature suggests chronic infection of genetically susceptible individuals with the food-borne zoonotic trematodes *Clonorchis sinensis* (*C sinensis*) and *Opisthorchis viverrini* (*O viverrini*) may play a role.

Observations: Although most infected people remain asymptomatic, untreated indolent infections with *C sinensis* and *O viverrini* may persist in peripheral intrahepatic bile ducts for almost 30 years. During this period, the trematodes' feeding activities and their excretory-secretory products may damage the bile duct epithelium and promote local inflammation. These pathological processes could then provoke epithelial desquamation, adenomatous hyperplasia,

goblet cell metaplasia, periductal fibrosis, and granuloma formation that are conducive to the initiation and progression of cholangiocarcinoma in genetically susceptible people. The International Agency for Research on Cancer has determined that there is sufficient evidence in humans for the carcinogenicity of chronic infections with *C sinensis* and *O viverrini*.

Conclusions: Timely serodiagnosis of indolent *C sinensis* and *O viverrini* infections is important as these parasites may be a risk factor for cholangiocarcinoma in veterans who served in Vietnam. About 774,000 living Americans served in Vietnam and there is an urgent need to develop sensitive and specific serologic assays to detect both acute and indolent infections. We posit that testing and treatment of high-risk populations could lead to earlier detection and treatment of cholangiocarcinoma, leading to improved overall survival.

Cholangiocarcinoma is a heterogeneous, highly aggressive cancer of the biliary tract epithelium with an overall 5-year relative survival rate of only 9%.^{1,2} Although surgical resection of localized, intrahepatic cholangiocarcinoma is associated with improved overall survival, most patients present with advanced disease not amenable to surgery due to a late onset of symptoms.² Recently, an increased incidence of cholangiocarcinoma has been reported in the United States.³ Although relatively rare in the US, cholangiocarcinoma is prevalent across large parts of Asia, including China, Vietnam, Thailand, South Korea, and Taiwan.²

RISK FACTORS

To date, risk factors for developing cholangiocarcinoma have not been elucidated.^{4,5} However, a growing body of literature suggests that chronic infection of genetically susceptible human subjects with *Clonorchis sinensis* (*C sinensis*) and *Opisthorchis viverrini* (*O viverrini*) plays a role.^{6,7} The life cycle of these food-borne zoonotic trematodes involves

eggs discharged in the stool of infected humans, the definitive host.^{6,7} In nature, these eggs are ingested by freshwater snails, the intermediate host, where they undergo several developmental stages to form cercariae. Once released from snails into the water, free-swimming cercariae come in contact and penetrate freshwater fish where they encyst as metacercariae. Infection of humans occurs by ingesting undercooked, salted, pickled, or smoked freshwater fish infested with metacercariae. After ingestion, metacercariae excyst in the duodenum and ascend the biliary tract through the ampulla of Vater. They then mature into adult flukes that reside in small- and medium-sized intrahepatic biliary ducts.^{6,7}

Although most infected people remain asymptomatic, untreated indolent infections with *C sinensis* and *O viverrini* may persist in peripheral intrahepatic bile ducts for as long as 30 years, which is the lifespan of the trematodes.^{6,7} During this prolonged period, *C sinensis* and *O viverrini* feeding activities and their excretory-secretory products may damage bile duct epithelium and promote intense

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local inflammation.^{6,7} Conceivably, these pathological processes could then provoke the epithelial desquamation, adenomatous hyperplasia, goblet cell metaplasia, periductal fibrosis, and granuloma formation that are conducive to initiation and progression of cholangiocarcinoma in genetically susceptible people.⁸ Accordingly, the International Agency for Research on Cancer (IARC) has determined that there is sufficient evidence for the carcinogenicity of chronic infections with *C sinensis* and *O viverrini* in humans and that chronic infections with these trematodes cause cholangiocarcinoma.⁹ The IARC concluded that chronic infections with *C sinensis* and *O viverrini* are carcinogenic to humans (Group 1).⁹

DIAGNOSIS

Presently, the diagnosis of *C sinensis* and *O viverrini* infection is based on microscopic identification and enumeration of the parasites' eggs in weighted stool specimens using a formalin-ethyl acetate sedimentation concentration technique.^{6,7} This approach requires a labor-intensive test that is conducted by an experienced technician. The test has low specificity and sensitivity because eggs could be confused with those of nonpathogenic intestinal flukes that are morphologically similar and because eggs are not present in feces during all stages of the infection. Although diffuse dilatation of intrahepatic bile ducts by screening sonography is used to diagnose clonorchiasis in endemic areas, it has low sensitivity, particularly in patients with low-level *C sinensis* and *O viverrini* infections.¹⁰

To address the current diagnostic gap, several enzyme-linked immunosorbent assays (ELISA) have been developed for the diagnosis of *C sinensis*, including monoclonal antibody-based (mAb) ELISA and indirect antibody ELISA.^{11,12} However, both have important limitations. The mAb ELISA detects only active infections while indirect antibody ELISA cross-reacts with other liver flukes.^{11,12} Taken together, these data illustrate the difficulties in diagnosing asymptomatic individuals with low-burden *C sinensis* or *O viverrini* infections by existing laboratory methods.

Timely serodiagnosis of indolent *C sinensis* and *O viverrini* infections is important because these parasites have recently been raised as a risk factor for cholangiocarcinoma in veter-

ans who served in Vietnam.¹³ The American War Library estimates that as of February 28, 2019, about 610,000 Americans who served on land in Vietnam or in the air over Vietnam between 1954 and 1975 are alive, and about 164,000 Americans who served at sea in Vietnam waters are alive.¹⁴ To that end, Pseudos and colleagues screened 97 US veterans who served in Vietnam and identified 50 who reported exposure to raw or undercooked fish while there.¹³ None had evidence of active *C sinensis* or *O viverrini* infection. Blood samples obtained from these veterans were analyzed for circulating *C sinensis* and *O viverrini* antibodies using an ELISA developed in South Korea and 12 blood samples tested positive for the trematodes. Imaging of extrahepatic and intrahepatic bile ducts was unyielding in all cases. One veteran diagnosed with cholangiocarcinoma had repeated negative tests. However, the results of this study were challenged by several experts in this field because the authors did not report the sensitivity and specificity of the ELISA assay used.¹⁵

Serologic testing of US veterans who served in *C sinensis* and *O viverrini*-endemic countries for indolent infections with these parasites is not recommended at present.¹⁵ Nevertheless, there is an urgent need to develop sensitive and specific serologic assays, such as ELISA tests with recombinant antigens, to detect both acute and indolent infections caused by each biliary liver fluke in the US, including in patients diagnosed with cholangiocarcinoma. We posit that testing and treatment of high-risk populations could lead to earlier detection and treatment of cholangiocarcinoma, leading to improved overall survival in the population at risk.

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Disclaimer

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