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REVIEW ARTICLE /REVISIÓN

Opisthorchis viverrini infection

Infección por *Opisthorchis viverrini*

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A B S T R A C T

Opisthorchiasis, caused by consuming infected raw cyprinid freshwater fish or contaminated water with *Opisthorchis viverrini*, is one of the most important risk factors for cholangiocarcinoma in endemic countries, such as Thailand, Laos, and Cambodia.[1,14] It has been stimated that 67.3 million people are at risk of acquiring an *O. viverrini* infection, and 10 million people are infected.[14] Nowadays, the gold standard for the diagnosis of liver fluke is the demonstration of eggs in stool samples, nevertheless, there are more diagnosis methods such as immunochromatography, ELISA and PCR test.[13] The diagnosis of cholangiocarcinoma in most of the patients is in stages III or IV, because most of the patients are asymptomatic or the diagnosis is incidental in a routine health check, that's the reason why it has a poor prognosis at 5 years.[12] The standard treatment is the praziquantel. People who don't tolerate praziquantel could be treated with tribendimidine, with less adverse effects.[14]

Keywords: Liver fluke, Cholangiocarcinoma, Freshwater snail, Cyprinid fish, Praziquantel.

R E S U M E N

La opistorchiasis, infección causada por el consumo de perscado de agua dulce del género *Cyprinid* o por la ingesta de agua contaminada con *Opisthorchis viverrini,* es uno de los más importantes factores de riesgo para colangiocarcinoma en países endémicos, como Tailandia, Laos y Cambodia. [1,14] Se ha estimado que 67.3 millones de personas están en riesgo de adquirir la infección, y 10 millones de personas se encuentran infectadas. Actualmente, el método diagnóstico de elección para la infección es la demostración de huevos in muestras de heces, sin embargo, existen otros métodos diagnósticos de utilidad como la inmunocromatografía, el ELISA y la PCR. [13] En la mayoría de los pacientes al diagnóstico, el colangiocarcinoma se encuentra en estadios III o IV, debido a que la mayoría cursan de forma asintomática o el diagnóstico se realiza de forma incidental posterior a un chequeo médico de rutina, esta, es la razón por la que el pronóstico es malo a 5 años. [12] El tratamiento de elección es el praziquantel, y como tratamiento alternativo se encuentra la tribendimidina, con menores efectos adversos.[14]

Palabras clave: Trematodo hepático, Colangiocarcinoma, Caracol de agua dulce, Pez ciprínido, Praziquantel.

INTRODUCTION

Ophistorchis viverrini is the etiologic agent of the ophistorchiasis, and one of the potential risk factors to cholecystitis, cholangitis and the worst consequence, cholangiocarcinoma in endemic regions. It's a trematode (liver fluke) of the family *Opisthorchiidae*, and genera *Amphimerus*.[3]

It has a complex life cycle that includes two intermediate hosts such as freshwater snail and the cyprinid fish, and different definitive hosts such as the human, dogs, cats, foxes, and bears.[3]

Most of the patients are asymptomatic, and they are inly diagnosed when the cholangiocarcinoma is in stages III or IV, and it's unresectable, or like an incidental diagnosis in a healthcare checkup.

EPIDEMIOLOGY

The global estimate of the number of people infected with liver fluke is 10 million people. (8 million in Thailand). *Opisthorchis viverrini* is known from continental Southest Asia, particularly in Thailand, Lao PDR, Cambodia, Southern Vietnam, Malaysia, Siangapore and the Philippines. (**Table 1**)

Table 1. Risk factors for *Opisthorchis viverrini* infection.
Tabla 1. Factores de riesgo para infección por *Opisthorchis viverrini.*

Risk factors	
Demographic factors.	Male
	Age younger than 24 years or older than 55 years
	Low level of education
	Fisherman[17]
	Rice Farmer[17]
	People who migrate from the northeast of Thailand have a 2.28-2.42-fold higher risk of infection than nonmigrants[8]
Environmental and geographic factors	Households with dogs and cats
	Absence of sanitation
Health behavior factors	Persistent smoking
	Consumption of alcohol
	Consumption of raw freshwater fish
	Contaminated drinking water
	Unsafe disposal of food waste
	Lack of knowledge about praziquantel treatment[17]

LIFE CYCLE

1. The embryonated eggs of *O. viverrini* are ingested by freshwater snails (*Bithynia siamensis, B. gioniomphalos and B. s. simensis*) which are the first intermediate hosts and in which it goes through an asexual reproductive cycle.
2. In the digestive tract of the freshwater snail, the embryonated egg becomes a miracidia, the miracidia penetrates the snail tissue and transforms into a sporocyst, then into a rediae and finally into a free-swimming cercariae, the duration from ingestion of eggs until release of the cercariae takes about 2 months.
3. The cercariae swims to find their second intermediate host, the cryprinid fish, in which attach to the skin and transform into a metacercariae.
4. The metacercariae becomes infective in about 1 month.
5. The definitive hosts acquire the infection by eating fermented, raw, or undercooked freshwater cyprinid fish containing the infective metacercariae, in which digestive duct transforms into a larva. The larva migrates into the biliary system.[8] The entire life cycle requires 4-4.5 months and worms have a lifespan of about 10 years.
6. Finally, the embryonated eggs are excreted with the host's feces, the worms lay about 2,000-4,200 eggs daily. [8] (**Figure 1**)

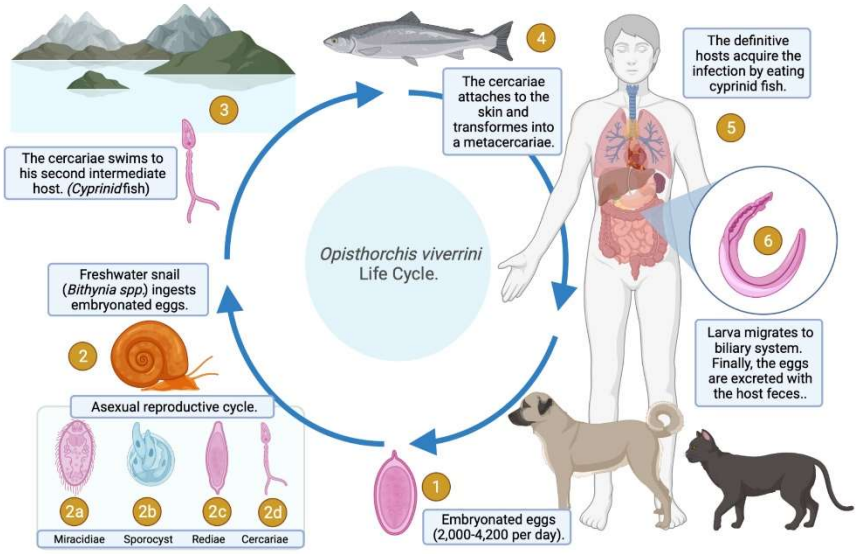

Figure 1. Life cycle of *O. viverrini*.
Figura 1. Ciclo de vida de *O. viverrini*.

PATHOGENESIS

O. viverrini is the most important risk factor to cholangiocarcinoma in endemic countries, nevertheless, the combination of exogenous carcinogens such as nitrosamines in fermented fish and pork magnify the risk of cholangiocarcinoma.[10]

The liver fluke suckers make a vicious cycle of ulceration (by clinging to the bile duct), inflammation and healing process those results in DNA damage and progression to cholangiocarcinoma.

O. viverrini causes an important immune response. One of the most important responses, is the interleukin-6 (IL-6), which is secreted in proinflammatory processes.

The IL-6 is associated with periductal fibrosis and promotes cell proliferation by activating progranulin expression.

The host defense system is activated by the O. viverrini's eggs antigens, which induce inflammatory cascade by upregulating TLR-4, activation of NFkB, inducible nitric oxide synthetase and cyclooxygenase-2; and expression and secretion of IL-6, IL-8, TNF-a, IL-B1 and oxidative stress reactants. [10] (**Figure 2**)

Macrophages activate by the egg's antigens generate an intense inflammatory cells infiltration and subsequently, granulomas around the eggs. [10]

One of the most interesting consequences of *the O. viverrini* infection is the different microbiome profile, *Helicobacter pylori* and *Opisthorchis viverrini* may potentially form an obligatory mutualism alliance. Both pathogens increase the degree of fibrosis, cholangitis, and duct hyperplasia.[10]

Cholangiocarcinoma is an aggressive cancer with high progression and metastasis, it's originated by chronic inflammation of the bile ducts in response to liver fluke infection.

The cholangiocarcinoma of the *O. viverrini* infection has different characteristic in contrast with primary sclerosing cholangitis and chronic inflammation's cholangiocarcinoma. [16]

In one study12, cancer cells characteristically present complex karyotypes with high aneuploidy, with different expression profiles and histological subtypes (adenocarcinoma, poorly differentiated (non-keratinized) and well-differentiated (keratinized) squamous cell carcinomas) of xenografted tumors.[16]

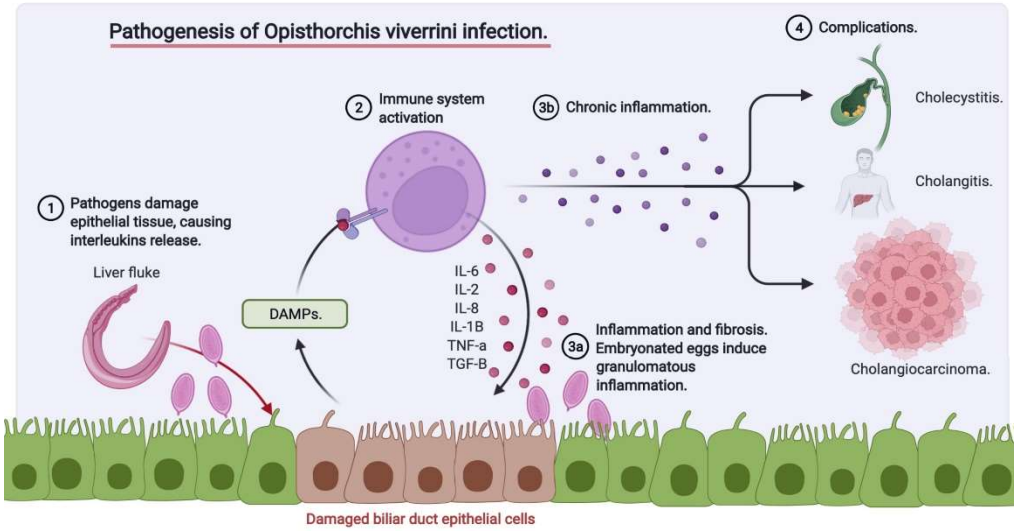

Figure 2. Pathogenesis of *Opisthorchis viverrini* infection. 1. The biliary ducts damaged release Damage-associated molecular patterns (DAMPs). 2. The DAMPs activate the immune system with the subsequent release of interleukins. 3. The chronic inflammation makes ulcers and fibrosis in the cubic epithelium. 4. The complications of *O. viverrini*'s infection are cholecystitis, cholangitis and cholangiocarcinoma.

Figura 2. Patogénesis de la infección por *Opisthorchis viverrini*. 1. El daño al epitelio cúbico de los conductos biliares ocasionan la liberación de patrones moleculares asociados a daño (DAMPs). 2. Los DAMPs ocasionan la liberación de interleucinas por activación del sistema inmune. 3. La inflamación crónica lleva a ulceración y fibrosis. 4. Las complicaciones asociadas a la infección por *O. viverrini* son la colecistitis, colangitis y el colangiocarcinoma.

CLINICAL MANIFESTATIONS

A major part of patients are asymptomatic or present unspecified manifestations before diagnosis, therefore, cholangiocarcinoma associated with this infection has a poor prognosis. Cholangiocarcinoma is the second most common primary liver cancer, after hepatocarcinoma, which derives from the epithelial cells of the bile ducts. Cholangiocarcinoma can be divided into 2 subtypes depending on the anatomical structure involved: extrahepatic (75%) and intrahepatic (25%), in turn, extrahepatic is subdivided into perihilar, middle and distal, depending on the location of the tumor. [10] (**Figure 3**)

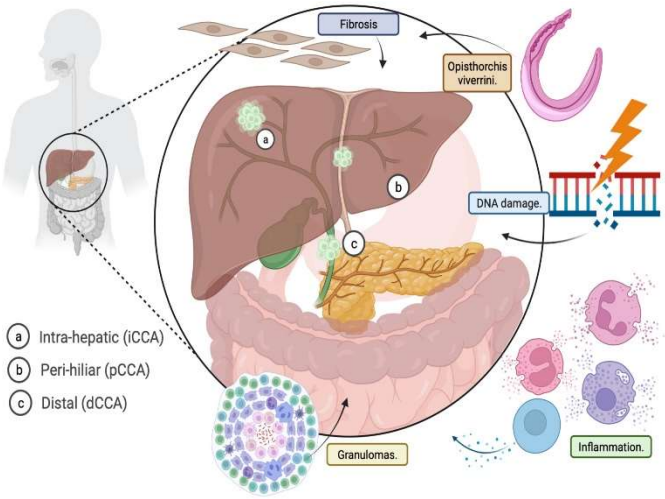

Figure 3. Cholangiocarcinogenesis. The *O. viverrini*'s infection produces chronic inflammation, biliary ducts granulomas, DNA damaged, and fibrosis, each of them, a necessary factor for cholangiocarcinoma. The cholangiocarcinoma is classified by its location, it could be intrahepatic (iCCA) or extrahepatic, which in turn is divided in perihiliar (pCCA) and distal (dCCA).

Figura 3. Colangiocarcinogénesis.

Extrahepatic cholangiocarcinoma can manifest with obstructive signs, such as pain in the right hypochondrium, pruritus, acholia, and coluria; and nonobstructive or systemic signs, such as fatigue, abdominal pain, weight loss, and liver mass on abdominal examination. [10]

Some patients are incidentally diagnosed on a medical check-up.

DIAGNOSIS

The diagnosis of *O. viverrini* infection is difficult, it needs a high grade of suspicious, because most of the patients are asymptomatic or it is diagnosed in a healthcare checkup. The gold standard continues to be the detection of eggs in feces, biliary fluid, or duodenal fluid, however, there are more diagnosis methods, with high sensitivity and specificity that can help us to diagnose the infection, such as immunochromatography, ELISA, etc. (**Table 2**)

Table 2. Diagnostic methods for *O. viverrini* infection and cholangiocarcinoma.
Tabla 2. Métodos diagnósticos para infección y colangiocarcinoma por *O. viverrini.*

Diagnostic method	Accuracy.				Characteristics
	Sensitivity.	Positive Predictive value	Specificity	Negative Predictive value	
Laboratory examination.	Direct simple smear: 12.4%. (7.5-18.9%)[1] Kato-Katz technique: 68.3%. (60-75.7%)[1] Detection by fecal parasite concentrator kit (FPCK): 32.4%. (24.9-40.7%)[1]			Direct simple smear: 68.5%. (63.7-73%)[1] Kato-Katz technique:85.7% (81.4-89.3%)[1] Detection by fecal parasite concentrator kit (FPCK): 73.8%. (69-78.2%)[1]	It's the gold standard for diagnosis of human live fluke infection, the sample could be feces, bile, or duodenal fluid.[10]
ELISA-based serodiagnosis.	100%.[13]	97.9%.[13]	98.3%.[13]	95.4%.[13]	Consists in the reaction of antibodies to an antigen of *Opisthorchis viverrini* in the sera of infectious patients.[13]
Immunochromatographic test (ICT) - based serodiagnosis.	94.6%.[13]	91.2%.[13]	89.7%.[13]	95.4%.[13]	It´s a speedy and simple technique by dropping the sample containing an analyte onto a test strip, that produces a result in 10 to 15 minutes.[13]
Stool-PCR amplification test of OvNad subunits.	OvNad1: 64%. OvNad2: 88%. OvNad4: 80%. OvNad5: 100%.[9]	OvNad1: 100%. OvNad2: 100%. OvNad4: 100%. OvNad5: 100%.[9]	OvNad1: 100%. OvNad2: 100%. OvNad4: 100%. OvNad5: 100%.[9]	OvNad1: 77.12%. OvNad2: 91%. OvNad4: 85.85%. OvNad5: 100%.[9]	NADH dehydrogenase subunits were designed from partial sequence of *Opisthorchis viverrini* mitochondrial DNA.[9]
Endoscopic Ultrasonography.	96%.[11]	60%.[11]	96%.[11]	60%.[11]	The endoscopic ultrasonography is a minimally invasive procedure that uses high-frequency sound waves to produce detailed images of hepatobiliary and pancreatic systems. [11]
Magnetic resonance cholangiopancreatography (MRCP).	95.35%.[11]	94.74%.[11]	93.18%.[8]	94.43%.[11]	The MRCP is a special type of magnetic resonance imaging exam that produces detailed images of the hepatobiliary and pancreatic systems, including liver, gallbladder, bile ducts, pancreas, and pancreatic duct. [11]
Endoscopic Retrograde Cholangiopancreatography (ERCP) with Histopathology.	Aspiration cytology: 42.1%. Brush cytology: 58.4%. Biopsy: 63.5%. Double-tissue sampling (DTS): 64.9%. Triple-tissue sampling (TTS): 85%.[4]	Aspiration cytology: 100%. Brush cytology: 100%. Biopsy: 100%. Double-tissue sampling (DTS): 100%. Triple-tissue sampling (TTS): 100%.[4]	Aspiration cytology: 100%. Brush cytology: 100%. Biopsy: 100%. Double-tissue sampling (DTS): 100%. Triple-tissue sampling (TTS): 100%.[4]	Aspiration cytology: 32.7%. Brush cytology: 50%. Biopsy: 54.8%. Double-tissue sampling (DTS): 62%. Triple-tissue sampling (TTS): 53.8%.[4]	The Endoscopic Retrograde Cholangiopancreatography (ERCP) is an endoscopic technique used to obtain tissue samples. This technique increments the sensitivity and specificity of the diagnostic method.[4]

TREATMENT

The treatment against the oncological liver fluke consists of two guidelines which are based on, a) the control of the transmission of cases with the eradication of this parasite and, b) the prevention of the progression of the pathology to reduce the number of cases of cholangiocarcinoma induced by infection caused by *Opisthorchis viverrini.*[2]

For a long time, praziquantel has been used as an antimalarial treatment in conjunction with health campaigns focused on promoting the consumption of properly cooked meat and fish as well as reducing the spread of fecal matter in the environment due to deposition at ground level, inadequate latrines, defective drainage, and irrigation of plantations with wastewater. All these measures showed a 57-64% decrease in cases, as well as a 3-5% decrease in prevalence. However, infection by *O. viverrini* does not provide protective immunity to a person, so they are still susceptible to reinfection.[7]

In endemic areas, both cases of reinfection and cholangiocarcinoma have been reported, which have been associated with the consumption of praziquantel. Due to its poor control, dependence on deworming, self-medication by the population, and easy availability of the antimalarial in pharmacies as it is an over-the-counter product.[2] Although the data suggest an increase in cases, there is a lack of evidence linking treatment with praziquantel and the appearance of cholangiocarcinoma. The available evidence in animal models suggests that the factors that increase the risk of cholangiocarcinoma a load of worms and substances secreted in the bile duct that cause liver damage as well as periductal fibrosis, while another model in hamsters with cholangiocarcinoma was treated with praziquantel presented a slow progression of this pathology. And those studies in humans infected with *O. viverrini* relate to alcoholism and the consumption of foods rich in nitrosamines as risk factors for the development of cholangiocarcinoma. [15,7]

Alternative treatment

In one study, (Sayasone S, Keiser J, Meister I, et. al.) The use of tribendimimidine (single dose 200 mg children and 400 mg adults) was shown to be inferior compared to praziquantel (50 mg/kg of weight and 25 mg/kg of weight, two doses) against infection by O. *viverrini (All children included in the study were* cured).[14]

Nowadays, tribendimidine could be used to treat *O. viverrini* infection in people who don't tolerate praziquantel.[6,14]

Despite other pharmacological alternatives, praziquantel is the first-line medication, as it is a drug with a short half-life, which is safe and effective against the parasite and other trematodes.[15]

The WHO recommends annual deworming in endemic regions with a prevalence greater than 20% and every two years in populations with a risk of less than 20%. Thanks to the use of this sanitary measure, a decrease in cholangiocarcinoma morbidity was found when praziquantel was used as the first choice for infection caused by *Opisthorchis viverrini*.[2]

PROGNOSIS

Most patients with cholangiocarcinoma are diagnosed at stages III and IV at the time of diagnosis. [7] Cholangiocarcinoma has a poor prognosis with a 5-year survival rate of approximately 5% in the type intrahepatic and 17% in the perihiliar and distal cholangiocarcinoma. [9]

Conflict of interest

The authors declare that they have no conflict of interest.

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