Review

Fish and Food-Fatale: Food-borne Trematode *Opisthorchis viverrini* and Cholangiocarcinoma

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Article info

Summary

Neglected Tropical Diseases (NTDs) are a group of communicable diseases with a long history with human beings. NTDs are the proxy of poverty since they affect those in low-income and extreme-poverty populations, as those populations lack access to proper health care, clean water, sanitary conditions, and hygiene. NTDs create losses for a nation that come from the health and the economic sectors as well since the costs of diagnosis, prevention, and treatment strain the national purse strings. One of the 20 different forms of NTDs on the list is food-borne trematodes, comprises of *Fasciola*, *Paragonimus*, *Clonorchis*, and *Opisthorchis*. Currently, it is estimated that food-borne trematodes can cause a devastating effect on mortality and morbidity. All of them are zoonotic, as humans become infected by ingestion of a second intermediate host, such as freshwater snails, fish, or water vegetables. *Opisthorchis viverrini*, one of the food-borne trematodes that can be found mostly in South East Asia regions, especially in the Mekong basin, is regarded as a group 1 carcinogen leading to cholangiocarcinoma (CCA). This study aims to present the updated review of *Opisthorchis viverrini* and CCA.
Keywords: Food-borne trematode; Neglected Tropical Diseases; *Opisthorchis viverrini;* Cholangiocarcinoma

Introduction

World Health Organization (WHO) stated that Neglected Tropical Diseases (NTDs) are a diverse group of infectious diseases, consisting of 20 diseases, mainly found in 149 tropical and subtropical countries (Fig. 1) (Mitra & Mawson, 2017). NTDs affect one-sixth of the global population, with the assumption that one in every six people will be affected by at least one type of NTDs. According to the WHO, the mortality and morbidity caused by NTDs are extremely high, as measured by disability-adjusted life years (DALYs). Globally, NTDs have resulted in about 534,000 fatalities and 57 million DALYs to date (Lin *et al*., 2022)

Neglected Tropical Diseases affect those who lack access to proper health care, clean water, sanitary conditions, and hygiene in low-income populations and even those who are extremely poor. NTDs create losses for a nation that come from both the health and the economic sectors as well, since the costs of diagnosis, prevention, and treatment place a strain on the national purse strings (Redekop *et al*., 2017; Hotez, 2020). Considerable population mobility contributes significantly to the transmission of NTDs thus

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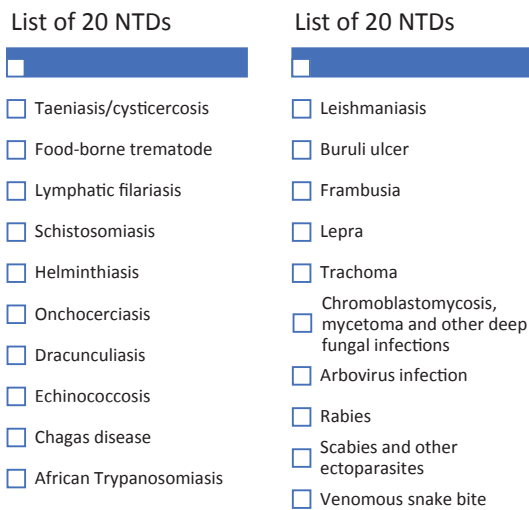| List of 20 NTDs	List of 20 NTDs
☐ Taeniasis/cysticercosis	☐ Leishmaniasis
☐ Food-borne trematode	☐ Buruli ulcer
☐ Lymphatic filariasis	☐ Frambusia
☐ Schistosomiasis	☐ Lepra
☐ Helminthiasis	☐ Trachoma
☐ Onchocerciasis	☐ Chromoblastomycosis, mycetoma and other deep fungal infections
☐ Dracunculiasis	☐ Arbovirus infection
☐ Echinococcosis	☐ Rabies
☐ Chagas disease	☐ Scabies and other ectoparasites
☐ African Trypanosomiasis	☐ Venomous snake bite

Fig. 1. List of 20 NTDs according to WHO.

more than a 70 percent of the cases are found in low-income or lower-middle-income countries. As a result of the increase in international traveling and climate change arising from global warming, NTDs are currently present in a number of European nations as well. Nonetheless, when the Covid-19 outbreak spread throughout the entire world, the global goals to fight NTDs has once again been disregarded (Adepoju, 2020; Tilli *et al.*, 2021). NTDs lead to significant illness and mortality in addition to societal ignominy and prejudice; however, NTDs are effectively managed, diminished, or eradicated through the intervention of a variety of efficient public health measure. The current most cost-effective interventions in global health are those aimed at eradicating and controlling NTDs. By 2030, point 3.3 of the Sustainable Development Goals (SDGs) on NTDs calls for the eradication of NTDs, malaria, tuberculosis (TB), and HIV/AIDS (Addisu *et al.*, 2019).

Distribution of liver trematodes

One of the 20 different forms of NTDs on the list is food-borne trematodes. The term "food-borne trematodes" refers to a collection of illnesses brought on by a number of trematode worms, including *Clonorchis, Opisthorchis, Fasciola, and Paragonimus.* Two intermediary hosts serve as a broad overview of this category of trematodes. Aquatic vegetables, tiny and medium-sized fish, water snails, and crustaceans are examples of the first intermediate host and the second intermediate host, which are generally unique from one another. Humans as the definitive host will be infected if they consume food that has been contaminated by the second intermediate host (Robinson & Sotillo, 2022). The total number of people infected around the world may be more than the 2 million DALYs and deaths that the WHO estimates are lost globally. Food-borne trematodes is thought to have infected over 75 million individuals worldwide as of today, with 0.2 million new cases reported year.

This information might not be accurate as there are many undetected cases (Chai & Jung, 2020).

The liver trematode worms in the *Opisthorchiidae* family that cause the food-borne trematode illnesses Opisthorchiasis and Clonorchiasis, which are *Opisthorchis viverrini* (*O. viverrini), Opisthorchis felineus* (*O. felineus*) and *Clonorchis sinensis* (*C. sinensis*). Over 700 million people globally, mostly in endemic regions of East and Southeast Asia, including Siberia, as well as numerous European countries, are at risk of acquiring one of these three tropical diseases (Suwannatrai *et al.*, 2018). *O. viverrini* is mainly restricted to Southeast Asia, *O. felineus* is indigenous to North Asia and Eastern Europe, and *C. sinensis* is prevalent in Korea, sections of Russia, China, and Vietnam (Sripa *et al.*, 2021; Pakharukova *et al.*, 2023). According to China's national parasite inquiry, the prevalence of Clonorchiasis has increased by about 0.37 percent since the initial examination in 1988, and a recent study found that the prevalence of C. sinensis infection is 29.3 percent (Gao *et al.*, 2020; Jiang *et al.*, 2021). While nearly 1.2 million people reside with *O. felineus* in Western Siberia and other regions of the Russian Federation (Ovchinnikov *et al.*, 2017). In addition, it is thought that about 10 million people in Southeast Asia are affected with *O. viverrini* (Sripa & Echaubard, 2017; Almanfaluthi *et al.*, 2022). This liver parasite poses serious public health issues in Thailand, Democratic People's Republic of Lao, Cambodia, and Vietnam (Thinkhamrop *et al.*, 2019a). There has been concern about these trematode worms in the western world due to reports of them in the United States (USA) as the surge in Asian immigrants or travelers, and the import of undercooked cyprinoid fish (Kaewkong *et al.*, 2013; Lu *et al.*, 2018). Additionally, some infections, like *Paragonimus westermani*, (*P. westermani*)can be acquired locally in the US where infected first and second intermediate hosts may be prevalent (Fried & Abruzzi, 2010).

Life cycle and morphology of *O. viverrini*

The *O. viverrini* life cycle starts when the embryonic eggs laid by the adult worm enter the bile duct and are eliminated in the feces. These embryonated eggs do not hatch if they are not introduced to fresh water bodies (small ponds, rivers, flooded rice fields, reservoirs, or rivers), since an intermediate host snail, such as the first intermediate host *O. viverrini*, will ingest them. The miracidia develop into sporocysts, rediae, and cercariae after hatching in the snail's digestive tract. Numerous thousands of cercariae are released every day as a result of snails' asexual reproduction. From the time eggs are consumed until cercaria are released, it takes roughly two months. The free-swimming parasites then look for the cyprinoid fish, which serves as their next intermediate host. They encyst in the fins, skin, and muscles of the fish and develop into metacercaria after 21 days. The bile duct is where the metacercaria attach after swallowing and develop into adults, who lay eggs 3 to 4 weeks later (Fig. 2). The metacercaria first exist in the duodenum before ascending through the ampulla of vater into the
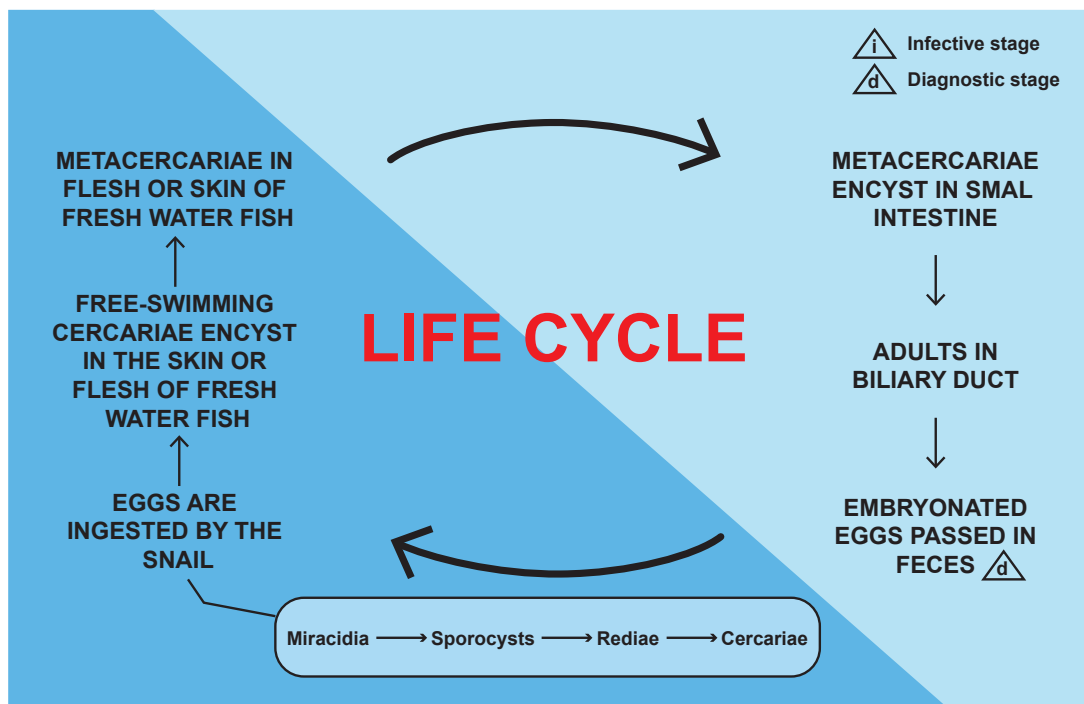
Fig. 2. Life cycle of *Opisthorchis viverrini*.

bile duct (Khieu *et al*., 2019; Suwannahitatorn *et al*., 2019; Tangka-wattana *et al*., 2021; Sota *et al*., 2022).

Opisthorchis spp. eggs are frequently indistinguishable from *C. sinensis* eggs, measuring 19 – 30 m long by 10 – 20 m wide. The egg has been operated on, and the opercular "shoulder" and abopercular knob are both noticeable. As they are passed through excrement, embryonic eggs (Fig. 3). Adult worms are flattened on the abdomen, approximately 7 mm (5.4 – 10.2) long by 1.5 mm (0.8 – 1.9), lanceolate, thin, and transparent. The bile ducts and pancreas of the mammalian host are where adult worms live and connect to the mucosa. They may reside in the pancreatic duct, gallbladder, or larger bile duct in cases of serious illness. They produce 2000 to 4200 eggs per day (3160 eggs in average) (Rach-prakhon & Purivirojkul, 2021a, Charoensuk *et al*., 2022). The exact life span of *O. viverrini* in human is not clearly known, however, it may be over than 25 years (Kaewkes, 2003; Petney *et al*., 2018)

Genetic diversity of *O. viverrini* (*O. viverrini* sensu lato)

There had formerly been some investigations of the genetic diversity of *O. viverrini*, and the first publication utilizing the isoenzyme analysis method appeared in 1993 (Sueblinvong *et al*., 1993). Since then, a number of molecular and genetic indicators studies have been carried out on *O. viverrini* using multilocus enzyme electrophoresis (MEE), nuclear and mitochondrial DNA sequencing, and microsatellite marker fragment analysis (Saijuntha, 2007; Kiatsopit *et al*., 2014; Pitaksakulrat *et al*., 2018; Namsanor *et al*., 2020).

According to a thorough molecular study in 2007, *O. viverrini* sensu lato (s.l.) is a species complex consisting of numerous cryptic species (genetically different species that share a similar morphology) found in different wetland systems in Thailand and Laos (Saijuntha, 2007). Bithynia snails serve as first intermediate vector, The first intermediate hosts in the life cycle are, a complex species that includes at least 11 cryptic species that are related to *O. viverrini* sl. As a result, both species complexes are highly genetically variable in relation to geography (Hughes *et al*., 2017).

Despite this, a later study in 2010 that used AMOVA to assess the populations rejected *O. viverrini* s.l. as a species complex since a study didn't find any differences that were significant. Six populations of *O. viverrini* from Thailand, Laos, and Cambodia were examined in this genetic study using a single marker and a portion of the ND1 sequence. The limited variation that has been noted in a number of prior research also makes it crucial to emphasize that mitochondrial DNA appears to be inappropriate and untrustworthy for systematic and population genetic studies of *O. viverrini* (Thaenkham *et al*., 2010). Numerous research looking at genetic diversity and population structures have employed microsatellite DNA markers to bolster the idea that *O. viverrini* was a species complex (Pitaksakulrat *et al*., 2017; Namsanor *et al*., 2020). In Bangkok, Thailand, most of the *Opisthorchis viverrini* s.l. cercariae were detected in the snail vector, *Bithynia siamensis siamensis* (Rachprakhon & Purivirojkul, 2021b). *O. viverrini* s.l. can be recognized from *C. sinensis* and *O. felineus* by differences in morphology, genetics, and geographic distribution (Saijuntha *et al*., 2014; Pakharukova & Mordvinov, 2022).

Fig. 3. The eggs of *O. viverrine* (A) and adult worms (B).
(Taken from cdc.gov/dpdx/opisthorchiasis/index.html)

Significance of *O. viverrini* infection in public health

O. viverrini can cause hepatobiliary abnormalities, such as cholangitis, cholecystitis, cholelithiasis, advanced periductal fibrosis, and lastly, cholangiocarcinoma as the end point. Due to its connection to recurrent neglected tropical diseases whose effects on public health are advised to be underestimated, food-borne trematodes have come under increased scrutiny. The greatest incidence of CCA in the world and a hotspot for *O. viverrini* transmission can both be found in northeast Thailand. In this region, the annual incidence of CCA ranges from 93.8 to 317.6 per 100,000 people, with the majority of cases ending in death within one to two years of diagnosis (Steele *et al*., 2018). The widespread nature of helminth infection is a significant epidemiological trait. This means that while most infected people have relatively low worm burdens, some have substantial worm burdens. The risk of sickness is typically higher in those with the most worms, who also contribute disproportionately to environmental pollution. In *O. viverrini*, the usual pattern of overspread has a long history. It has been hypothesized that environmental exposure (host behavior shows considerable age dependence), individual vulnerability (genetic and immunogenic variables), and social factors are to blame for the overabundance of helminths (Echaubard *et al*., 2016).

The *O. viverrini*'s complex life cycle, which involves a number of hosts and surroundings, makes it challenging to control using traditional techniques. Poor hygiene and sanitation may serve as the one of main factor resulting in stool contamination to water bodies, hence the reservoir hosts such as stray or domestic dogs and cats may also contribute to the transmission of this fluke. (Sripa *et al*., 2017; Tangkawattana & Sripa, 2018).

Risk factors of *O. viverrini* infections

The persistence of *O. viverrini* infection in endemic regions is influenced by a diverse array of risk factors (Suwannatrai *et al*.,

2018). Age may be a factor in the increased burden of infection among working-age individuals, as well as the higher incidence of infection among men and those with lower levels of education. Cultural activities related to fishing, food preparation, and eating raw fish, which are deeply ingrained as parts of the region's native rice fish culture, are the major factors contributing to the high rate of *O. viverrini* infection in the endemic area (Prakobwong & Suwannatrai, 2020). Consuming freshwater fish that has already been salted, or smoked that is either raw or undercooked and has metacercaria on it can infect humans or other fish-eating animals. It is just the consumption of raw fish which caused the infection of *O. viverrini*, meanwhile the consumption of fish sauce doesn't have any impact as the fish sauce has undergone further process from the raw fish into fish sauce as the end product (Thaewnongiew *et al*., 2014).

Food sharing among household and neighbor is a common practice in communities of local inhabitants (Isaan people) in Northeast Thailand, as this province bear the heaviest burden of *O. viverrini* infection (Phimpraphai *et al*., 2018). Sharing raw fish dishes among neighbors may increase the prevalence of infection but also raise the danger of inflammation-related diseases because the average per-person infection intensity will be lower. Food sharing was tightly and favorably correlated with eating raw fish frequency and the dishes consumed variation (Saenna *et al*., 2017). This implies that raw fish dishes are more likely to be accepted, offered, and consumed in households with higher food sharing propensities. Comparatively to homes that do not exchange food or do so only to a limited extent, this raises the risk of transmission. The smaller, less common raw fish is typically used in these communal dishes. The type of fish that is potentially infected with "*pla khao noi*" (Isan-Lao for small cyprinid fish) which is widely used in processing raw fish is usually found in local villages to be sold cheaply or distributed free of charge to fishermen's families and friends. Other factors that promoting the everlasting transmission of *O. viverrini* including occupation factor where rice farmers

and fishermen are more likely to consume raw fish dishes and defecate in or near wetlands and rice fields due to their daily activities. As praziquantel (PZQ) is easily obtained through pharmacy in Thailand, local people are taking this drug by themselves without proper medical supervision every time they get reinfection with *O. viverrini*, and this phenomenon eventually leading to drug resistance. Saengsawang *et al*., revealed that the vicious cycle of *O. viverrini* reinfection and repeated usage of anthelmintic PZQ obviously increasing the risk of CCA (Saengsawang *et al*., 2016; Suwannatrai *et al*., 2018; Thinkhamrop *et al*., 2019b).

To overcome the continuous transmission of *O. viverrini*, a novel control method known as the "Lawa model" employing the Eco-Health / One Health approach was implemented to the Khon Kaen Province's in Lake Lawa area, Thailand where liver flukes are endemic. The program comprises anthelmintic medicine therapy, innovative approaches to intense health education in the community and in schools, ecosystem monitoring, and engaged community involvement. Due to its success stories, this technique began in 2007 and has continued for more than ten years, as this control method has been adopted by WHO (Sripa *et al*., 2017).

The incidence of cholangiocarcinoma caused by liver trematodes

Fish-borne trematode infection and CCA have a well-established link. This infection can result in hepatobiliary diseases such as hepatomegaly, chronic cholangitis, cholecystitis, cholelithiasis, periportal system fibrosis, gallstones, and bile duct cancer or cholangiocarcinoma (CCA) (Sripa *et al*., 2021). The World Health Organization's International Agency for Research on Cancer (WHO) categorized *C. sinensis* and *O. viverrini* as a Group 1A carcinogen (a metazoan parasite harmful to humans). It is estimated that 100 per 100,000 in men and 40 per 100,000 in women or approximately 1 % of the invidual with opisthorchiasis would develop into CCA

and the disability-adjusted life years (DALYS) caused by the infection of *O. viverrini* and CCA as high as 150 – 235 × 103 DALYs (Saijuntha *et al*., 2019b, Brindley *et al*., 2021a, Liau *et al*., 2023). Despite not being officially recognized as a human carcinogen, *O. felineus* is a member of Group III has potentially carcinogenic (Banks *et al*., 2012; Saltykova *et al*., 2018; Tantrawatpan *et al*., 2020). In recent publication by Fedorova et al, they stated that a positive relationship between infection of *O. felineus* with the risk for CCA in the hospital-based study in Westen Siberia, as the CCA patients had the history of *O. felineus* infection. This study revealed that this species might hold the possibility to be the Group 1A carcinogen, as the *O. viverrini* and *C. sinesis (Fedorova et al*., 2023).

In comparison to *O. felineus* area, long-term observation study indicated that CCA is more frequently documented in *O. viverrini* and *C. sinensis* endemic regions. Even though the carcinogenesis pathology caused by parasitic infection may be driven by a variety of events known as chronic inflammatory infectious processes, helminthiases related to malignancy remain mainly unknown. Additional pathways involve genomic instability, induction of invasion, angiogenesis, and cancer metastases, deregulation of tumor suppressor genes, prolonged cell growth, cell death prevention, energy metabolism regulation, and immune evasion (Machicado & Marcos, 2016; Pakharukova *et al*., 2019; Fedorova *et al*., 2020). The three liver trematode species differ in how they become carcinogens, but there have been no comparative research studies, thus the information that is currently accessible is not sufficiently clear.

The role of parasite induces cholangiocarcinoma

Depending on its cellular source, hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are the two subtypes of primary malignant liver cancer that are distinguished histologically (El Jab-
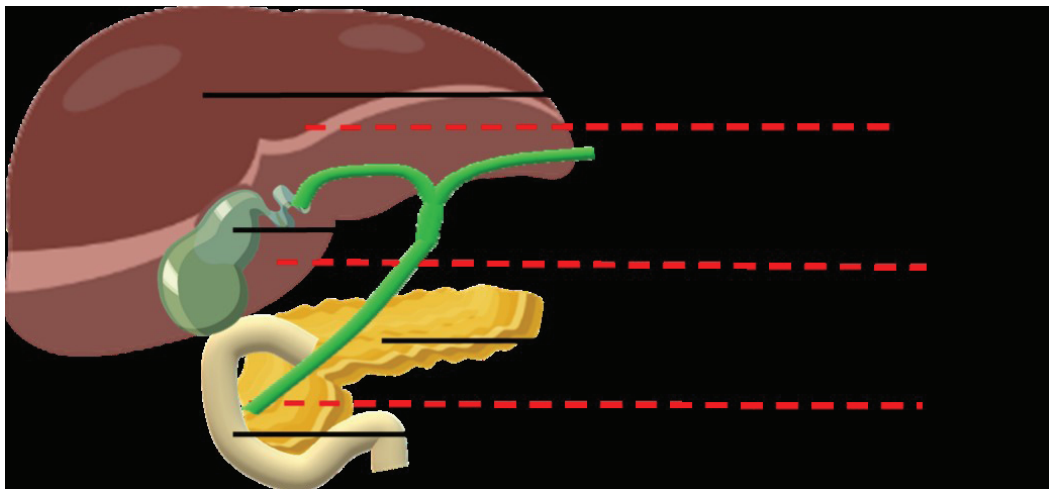

Fig. 4. Classification based on anatomical location of cholangiocarcinoma.

bour *et al*., 2019). Hepatocellular carcinoma responsible for nearly 75 and 80 percent of cases worldwide. The rarest type of cancer is by far CCA constituting 10 to 25 percent of primary hepatic cancers. However, liver cancer incidence reveals 15 times higher in East Asian regions where Opisthorchiasis and/or Clonorchiasis are endemic. The fatality and morbidity of CCA are directly proportional and therefore have a shabby prognostic. The most prevalent kind of CCA is extrahepatic estimated 80 – 90 % and about 50 % of all CCA are perihilar (affecting the main branches of the ducts). The 5-year survival rate for CCA patients after diagnosis has not increased is not good, as low as 5 % to 17 % of total cases (Chansitthichok *et al*., 2020).

Cholangiocarcinoma can develop based on the anatomical location into intrahepatic (iCCA) or extrahepatic, while extrahepatic CCA are divided into perihilar (pCCA), and distal (dCCA); those may appear differently in terms of clinical findings, pathology, and epidemiology (Fig. 4) (Pratap *et al*., 2018; Khan *et al*., 2019; Singal *et al*., 2020).

Various iCCA-specific risk factors are associated, although it is unclear how they cause iCCA to evolve. The prevalence of CCA varies globally due to variations in genetic and environmental risk factors. The liver fluke infestation is pathogenically linked to CCA throughout Asia, especially in regions where *O. viverrini , Clonorchis sinensis* and kindred flukes is endemic (Saijuntha *et al*., 2019a). A sizable previous amount of epidemiologic evidence supports the involvement of liver flukes in the emergence of CCA, particularly *Clonorchis sinensis* (Chinese liver fluke) and *O. viverrini*, both are significantly related to malignancy. Opisthorchiasis infection has been linked to an elevated risk of 2 to 5 times developing intra and extra-hepatic CCA as a result of adult flukes residing in the biliary tract over years and triggering an immunological response from the host and inducing prolonged inflammation (Massarweh & El-Serag, 2017).

Pathogenesis of *O. viverrini* induced cholangiocarcinoma

The mechanism of *O. viverrini* induced CCA is uncertain, but chronic inflammation is thought to play a role as results from the flat worm infecting the host's biliary tract. (Chaiyadet *et al*., 2015; Zimmermann, 2019). The most frequent cause of CCA is malignant transformation of cholangiocyte cells, while it is also possible for peribiliary gland epithelial cells and/or biliary stem cells to evolve into cancer. Moreover, hepatic stem/progenitor cells are confirmed to be the source of some subgroups of CCA and combined HCC/CCA (Nakagawa *et al*., 2018). There are at least three different pathways embroiled in the opisthorchiasis driven carcinogenesis. First, once the parasites mechanically injured the biliary epithelium, the tissue healing has begun. In order to build a cellular covering for the injury, tissue healing reactions cause the release of enzymes that breakdown the extracellular matrix, impede with epithelium adhesion, and stimulate cell proliferation. Normally, a sum of released substances reacts to cease the process after the injury has healed. Healing and recovery from injury is hampered by chronic epithelial breakage caused by the grazing and passage activity of the liver fluke. DNA damage and oncogenesis are presumed to be caused by the continuous cell division involved with tissue injury that is mechanically driven in the presence of external co-factors. The wound - healing reaction apparently causes cell proliferation, which, as in existence of co-factors, considerably aids in the growth of cancer (Sripa *et al*., 2018b, Sawanyawisuth *et al*., 2021).

Chronic immunological responses are the second pathway that can increase the risk of malignancy. During cholestasis and inflammation, it is discovered that the quantities of growth factors, cytokines, and bile acids are enhanced. These substances contribute to these molecular changes and promote the proliferation and viability of changed cells. The immune reaction is initiated when Nitric Oxide (NO) and oxygen radical spurt from activated phagocytes often eradicate various antigens. This reaction may have unfavorable harmful side effects as NO is carcinogenic and induces endogenous carcinogenic nitrosamine production (CCA primary catalyst) (Smout *et al*., 2011; Chaiyadet *et al*., 2015).

The immune reaction in those with opisthorchis infection unaffecting the invading worms resulting in a protracted reaction and NO buildup. Inducible nitric oxide synthase (iNOS) is upregulated in inflammatory cholangiopathies and CCA, where cytokines promote iNOS production in epithelial cells. Plasma and urine nitrite contents increase although nitrosamine and their precursors' contents from diet are not rising in opisthorchis infection. As the excretory metabolite of nitrosamines, nitrites are a good indicator of significant endogenous nitrosamine synthesis. The eradication of the parasite, which is easily achieved by using the anthelmintic medication praziquantel, should make this fluke infection outcome reversible (Jaiswal *et al*., 2001; Smout *et al*., 2011; Sripa *et al*., 2018a).

Further research established that the areas of fluke-induced tumorigenesis contain DNA breakage caused by nitrite generation ((Kawanishi *et al*., 2006). Oxidative DNA breakage and nitrates including 8-oxo-7,8-hydro-2'-deoxiguanine (8-oxodG) and 8-nitroguanine (8-NG), both have been connected to inflammation-related tumorigenesis. Nitric oxide (NO) and reactive nitrogen oxide species (RNOS) synthesis are known to interact with cytoplasmic DNA and proteins, both of which are the results of increased inducible nitric oxide synthase (iNOS) activity. Additionally, interaction between reactive nitrogen oxide species (RNOS) and cellular genome trigger in DNA strands breakage and transmutation. Nitric oxide (NO) and RNOS as well interact with DNA repair enzymes which is promptly rendered inactive by S-nitrosylation of the cysteine residue in its active site, to further enhance mutagenesis. During opisthorchiasis elicited an inflammatory response and released ROS/RNS with high oxidative potential. Afterward, biomolecules in the inflammatory region, including *O. viverrine* in the bile duct, will react with ROS/RNS. Opisthorchiasis has also been linked to higher rates of nitrite and nitrate which are indicators of

endogenous NO production. A study linking urinary nitrite levels and infection severity with a higher risk of CCA, demonstrated that nitrosamines are involved in the pathogenesis of opisthorchiasis. Nevertheless, the source of the rise in nitrite content is unknown, which may be related to modifications in the host's endogenous nitrite synthesis or a rising in external nitrites from fermentation products (Jaiswal *et al*., 2000; Vale *et al*., 2013) Long-lasting inflammation can also come from the eggs that the flukes lay and potential for cancer, even has not been thoroughly investigated, it may considerably impact toward disease as observed in the other primary cancer-causing fluke.

Finally, various soluble proteins and other mediators that fulfill numerous of function at the host-parasite boundary are excreted and secreted by liver fluke to endure for an extended of time in a hostile environment (Drurey & Maizels, 2021). It has long been assumed that this interaction affects host cellular homeostasis and aids in the development of cancer. In vitro studies demonstrated that the ES product of *O. viverrini* to mammalian cell lines causes morphological changes because the ES product is mitogenic and can be absorbed by host bile cells. This finding is supported by in vivo studies, the growth of CCA in a hamster opisthorchiasis model was faster when the model was given ES products before being infected with *O. viverrini* indicating that immunological memory can speed up carcinogenesis (Pakharukova *et al*., 2022).

Diagnosis and management of Opisthorchiasis

Diagnosis
O. viverrini infection diagnosis can be quite challenging because the majority of chronic human cases show few distinct signs or symptoms, with the exception of a palpable rise in liver frequency. Even in severe infections, hematologic and biochemical characteristics are typically unremarkable. Although ultrasound is not frequently used in the community, it can be useful in making the diagnosis since it can detect abnormalities in the gallbladder, such as muddy and irregular gallbladder walls, due to *O. viverrini* infection (Mairiang *et al*., 2012). The most accurate way to determine *O. viverrini* infection is to look for eggs in the bile, duodenal fluid, or feces/stool. Stool samples are examined for parasites, which is a common practice that provides a regular diagnosis. However, sensitivity and accuracy depend on the technique used for the fecal inspection and the microscopist's skill. The community-level persistence of *O. viverrini* infection is also a result of this constraint (Charoensuk *et al*., 2019).

Another diagnosis methods, such as serological, antigenic, molecular tests have been developed, but those methods are not for the regular usage. Using soluble excretory-secretory antigen from adult *O. viverrini* flukes, an immunochromatographic test kit based on ELISA principles was created, and this test kit showed a promising future with more than 9 % of sensitivity and specificity (Sadaow *et al*., 2022). Another test kit by using soluble somatic antigens from the adult *O. viverrini* worms with IgG and IgG4

conjugates, also have been introduced, by this test kit has higher specificity, but low sensitivity (Phupiewkham *et al*., 2021). Yet the serological detection methods are promising diagnostic, those methods carry some flaws, such as inability to distinguish the past or current active infection.

Antigenic diagnostic using urine antigen from the *O. viverrini* infected patient by using enzyme-linked immunosorbent assay (ELISA) has been proved to be effective in term of additional method accompanied with fecal microscopy test. This urine antigenic test can detect the infection before the treatment with PZQ until 4 weeks after the treatment, concluding that urine antigen could be a helpful test to determine the current active infection and also for effective PZQ treatment screening in community (Worasith *et al*., 2020).

Molecular methods including PCR and loop-mediated isothermal amplification (LAMP) have been explored. The LAMP targeting internal transcribed spacer 1 (ITS1-LAMP) with the fecal samples done has been used extensively for trematode infection diagnostic, but it has moderate specificity (61.5 %) yet high sensitivity (100 %). Eventually, the LAMP technique has higher sensitivity than conventional PCR method, but the limitation of this test is the ability of ITS-1 LAMP to amplify the *O. felineus* DNA set also (Arimatsu *et al*., 2012).

PCR method using 3 genes as targets, which are internal transcribed spacer-2 (ITS-2), cytochrome oxidase subunit 1 (cox1), and cytochrome b (cyb) by using fecal samples has been applied for screening or monitoring usage due the high sensitivity and specificity value. Using stool samples for PCR is one advantage as this test is not invasive, but this technique needs a skillful person and more expensive than other tests, limiting this method for routine usage (Liau *et al*., 2023).

Management
Praziquantel is an oral anthelmintic medication used to treat Opisthorchiasis. The recommended dosage for adults is 75 mg/kg taken orally for two days, split three times daily. The same dosage applies to children (CDC – National Center for Health Statistics – Homepage. https://www.cdc.gov/nchs/. Accessed on June 22, 2023). Food safety is a high priority for disease control due to tradisional habits in the impacted regions. Prevention strategies for the disease include programs to raise awareness of food safety, a better approach for identifying and treating opisthorchiasis, and school-based health program for longer-term goals.

Diagnosis and management of Cholangiocarcinoma

Diagnosis
The diagnosis of CCA is frequently complicated and necessitates a combination of several diagnostic techniques to pinpoint the stricture's anatomical location, differentiate within malignancy and benign strictures, define CCA coming from mixed hepatocellular-CCA, the bile or other primary hepatic tumors. As addition, the diagnosis of CCA must take into consideration of the tumor's

stage and grade in order to determine the best course of therapy (Brindley *et al*., 2021b, Shin *et al*., 2023). Until nowadays, CCA is confirmed by the patient history taking, clinical and laboratory (tumor biomarkers) also imaging diagnosis. As a result, numerous efforts have been undertaken to develop novel CCA biomarkers that could help with diagnosis as well as forecast prognosis and therapeutic response.

There are several biomarkers that can be used for the diagnosis of cholangiocarcinoma (CCA) depends on the type of samples. Talking about the samples from serum, carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) are the most useful biomarkers in the since both of these biomarkers have good sensitivity and specificity in clinical usage (Tshering *et al*., 2018). Biomarkers used from the tissue samples include gene methylations and DNA-related markers. It is important to highlight, however, that no one biomarker can be used alone to accurately diagnose CCA. Combining these possible biomarkers with other laboratory procedures is likely to considerably improve diagnostic accuracy.

Various investigations indicated possible non-invasive biomarkers for cholangiocarcinoma (CCA) found in body fluid, such as blood (serum), bile, urine and or other body fluids which are used until currently, such as:

1. Carbohydrate antigen (CA): CA19-9 is an antigen found in normal epithelial cells, including biliary ducts. CA 19-9 (carbohydrate antigen 19-9) is a cell surface glycoprotein complex that has been linked to pancreatic ductal adenocarcinoma (PDAC), and routinely used as tumor-marker in CCA. Elevated levels of CA19-9 correlate with tumor mass and degree of tumor differentiation. (Liang *et al*., 2015; Lee *et al*., 2020). Several studies found out that combination of CA19-9 with other biomarkers increasing the sensitivity and specificity. Study by Lapitz A, et al revealed that combination of serum extravesicles (EVs) contain protein biomarkers with CA19-9 has remarkable outcomes, as the sensitivity and specificity is higher compared than only CA 19-9 alone (Lapitz *et al*., 2023). However, CA19-9 is not specific to CCA and tumor of hepatobiliary, but can also be elevated in another tumor outside the gastrointestinal tract is one of the limitations. Another condition such as inflammatory condition or obstruction of biliary and respiratory tracts may also result in the elevation of CA19-9 level (Lee *et al*., 2020). Another limitation of CA19-9 as this biomarker has low positive predictive value, thus not useful for screening purpose.

2. Carcinoembryonic antigen (CEA): CEA is oncofetal glycoprotein produced by the gastrointestinal system (colon) that has been linked to tumor metastasis. This biomarker has high sensitivity and specificity, like CA-19-9. Ample researches have been published that highlight the diagnostic value of elevated CEA levels in bodily fluids in primary malignant tumors of the stomach, colon, biliary system, and pancreas (Joo *et al*., 2003; Loosen *et al*., 2017). However, like CA19-9, CEA is not specific to CCA and can be elevated in other types of tumors, and CEA has low sensitivity and specificity as prognostic marker (Kim *et al*., 2021).

3. Micro-RNA (miRNA): miRNA is small non-coding RNA that regulate the gene expression, as nowadays have been explored extensively as biomarkers of tumor, as miRNAs can circulate throughout the whole body, making these molecules obtainable in wide range of body fluid. miRNAs can play dual role, either as protective or oncogenic factors to tumor. The level of miRNAs varies, either increasing or decreasing, according to the tumor types. The level of several miRNAs, such as miR-26a, miR-141, miR-210, miR-31, miR-21, and miR-421 are increasing in CCA, through cell proliferation modulation (Haga *et al*., 2014). The accuracy of miRNAs as potential biomarker with another biomarker has also been studied. miRNAs-150, expressed in immune cells, such as B and T cells, if combined with CA19-9 results in more accurate CCA diagnosis. Level of CCA-19 inversely proportional with miR-NA-150, as CCA 19-9 level is high in CCA, whereas miRNA-150 level is low (Salem *et al*., 2020).

4. Long non-coding RNA (l (lncRNA): lncRNA is type of non-coding RNA with more than 200 nucleotides, which can be found inside in the EVs that circulate through human body. Like miRNA, lncRNA also play dual role, as tumor suppressor role or oncogenic role. Some specific lncRNAs that have been studied in relation to Cholangiocarcinoma, such as Prostate cancer-associated transcript 1(PCAT1), Neighboring enhancer of FOXA2 (NEF), Metastasis-associated lung adenocarcinoma transcript 1(MALAT1), H19, Colon cancer-associated transcript 1 (CCAT1), and ZEB1 Antisense RNA 1(ZEB-AS1) (Yang *et al*., 2020; Wu *et al*., 2022).

5. Exostosin1 (EXT1): EXT1 is a gene encodes an endoplasmic reticulum-resident type II transmembrane glycosyltransferase involved in the chain elongation step of heparan sulfate biosynthesis. This gene is believed as tumor suppressor gene and has been investigated as a potential biomarker for CCA. However, its diagnostic accuracy and clinical utility are still being evaluated (Zeng & Tao, 2015).

It is important to note that while these biomarkers have been studied for their potential in diagnosing CCA, none of them have been considered adequate for clinical application on their own. Combination of biomarkers and further research are needed to improve the accuracy of CCA diagnosis. The main challenge in the short term of the effective biomarkers of CCA is not to discover new molecules and pathways, but rather to validate or refute the role of current methods in bridging the gap between research findings and clinical application. This suggests that the focus should be on validating existing biomarkers and study protocols to facilitate the translation of research into clinical practice.

Recent breakthroughs in "Omics" technologies provide great prospects for the identification of biomarkers connected to diseases. Next-generation biomarkers for cholangiocarcinoma offer several potential benefits, including: 1) Early and non-invasive diagnosis as next-generation biomarkers have the potential to improve the early detection of cholangiocarcinoma, allowing for timely intervention and treatment. This can significantly contribute to reducing

mortality rates associated with the disease; 2) Improved patient outcomes by providing more accurate and reliable diagnostic information and also next-generation biomarkers can help guide treatment decisions and improve patient outcomes. They can aid in identifying patients who may benefit from potentially curative approaches, such as surgery; 3) Prognostic and predictive capabilities since next-generation biomarkers have the potential to provide valuable prognostic and predictive information. They can help determine the likely progression of the disease and predict the response to specific therapies, enabling personalized treatment strategies; and 4) non-invasive monitoring, as these biomarkers can also facilitate non-invasive monitoring of disease progression and treatment response. This reduces the need for invasive procedures, such as tumor biopsies, and allows for more frequent and convenient monitoring of patients (Silsirivanit *et al*., 2014; Chang *et al*., 2020; Rodrigues *et al*., 2021).

Management

Management of CCA differs according to the anatomical location of the disease, similar to how it is diagnosed. iCCA frequently receives therapy with surgical resection and estimated 60 percent of patients continue to live for five years after the procedure. But the majority of iCCA patients have big, incurable tumors when they first show. For patients in the beginning phases of pCCA or dCCA, surgery is also a choice. Surgery combined with neoadjuvant chemoradiation is the sole curative treatment for pCCA attended by liver transplantation. The overall five-year survival rate of pCCA patients who receive surgery is approximately 10 % - 40 %. While pCCA patients who received neoadjuvant treatment attended by liver transplantation survived for 5 years around 65 percent. The management of dCCA is also surgical resection, yet the Whipple operation technique is often used. After surgical, a mere 27 percent of patients remain alive for five years (Brindley *et al*., 2021b, Bao *et al*., 2022). Additionally, a preventative program for undiagnosed populations with initial-stage CCA is necessary to undergo surgery. The only effective treatment for CCA is a resection surgery (Rizvi & Gores, 2013; Alsaleh *et al*., 2019; Pakharukova *et al*., 2019).

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Ethical Approval and Conflict of Interest

This review article is not accompanied by an ethical statement because it does not involve humans or animals as research subjects. All of the authors have read and agree with the manuscript. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors Contribution

ST and MM both contributed equally in all of the process, from conceptualization, drafting, writing, review and editing.

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