Endoscopic Management of Biliary Fascioliasis and Identification of Fasciola Fluke Based on Mitochondrial DNA: A Case Report from Thailand

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Abstract. A patient from western Thailand with biliary fascioliasis was admitted at Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The patient presented with right-upper-quadrant abdominal pain with no history of jaundice. Laboratory results showed eosinophilia. Ultrasonography and computerized tomography revealed biliary dilation in the intrahepatic and extrahepatic bile ducts, with duct wall thickening, but these observations did not help clarify the differential diagnosis. Endoscopic retrograde cholangiopancreatography (ERCP) showed dilatation of the common bile duct caused by obstruction from a fluke. After removal of the fluke and treating with triclabendazole, the symptoms disappeared and the laboratory values returned to normal. Morphological identification of fresh and carmine-stained fluke and DNA sequencing of mitochondrial cytochrome c oxidase subunit I gene identified the fluke as Fasciola gigantica. This report endorses the diagnostic and therapeutic role of ERCP in patients who present with right-upper-quadrant pain without jaundice caused by fascioliasis.

Keywords: Fasciola gigantica, biliary fascioliasis, endoscopic retrograde cholangiopancreatography, mitochondrial cytochrome c oxidase subunit I gene

INTRODUCTION

Fascioliasis is a waterborne and foodborne zoonotic disease caused by two parasites of class Trematoda, genus Fasciola: F. hepatica and F. gigantica (Mas-Coma et al., 2009). Worldwide, it is one of the most common parasitic diseases in cattle and buffaloes. F. hepatica typically occurs in temperate regions, except Oceania, while F. gigantica is widespread in tropical areas of South and Southeast Asia, and Africa (Youn, 2009). In Thailand, a report in 1991 showed that up to 85% of host animals are infected, mainly with F. gigantica (Srihakim and Pholpark, 1991). The prevalence is high in lowland areas and near water reservoirs, such as dams and large ponds, in which Lymnaea auricularia rubiginosa, the intermediate snail host of F. gigantica, is found (Viboolyavatana, 1981).

Humans are accidental hosts, becom-
ing infected by ingesting raw green vegetables or drinking water contaminated with encysted metacercariae. According to the WHO, it is estimated that at least 2.4 million people are infected in more than 70 countries worldwide, with several million at risk (WHO, 2017). It is likely that whenever animal cases are reported, human cases also exist (Furst et al, 2012). The first case of human fascioliasis in Thailand was reported in 1967 (Tesana et al, 1989): two patients from northeastern Thailand, presenting with cholecystitis and gall stones, identified with *F. gigantica* infection. Since 1990, at least 25 sporadic cases have been reported in Thailand, mainly caused by *F. gigantica*, with a few cases of infection with *F. hepatica* (Aroonroch et al, 2006).

Identifying *Fasciola* flukes based on morphological criteria is not accurate, because of the morphological diversity within the species (Lotfy et al, 2002; Valero et al, 2012). Several studies have used DNA-based approaches to identify intraspecific phylogenetic relationship of *Fasciola* spp by sequencing various DNA regions, viz. internal transcribe spacer 1 (ITS1), ITS2, 28S rDNA, mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit I (NDI) gene and cytochrome c oxidase subunit I (COI) gene (Adlard et al, 1993; Itagaki and Tsutsumi, 1998; Marcilla et al, 2002; Itagaki et al, 2005a,b; Ichikawa et al, 2010; Rokni et al, 2010; Chaichanasak et al, 2012).

In general, clinical diagnosis of fascioliasis is difficult, although it is often associated with a history of ingesting freshwater plants and having fever, right-upper-quadrant pain, or intrahepatic cystic lesions, together with absolute peripheral eosinophilia (Aroonroch et al, 2006). Endoscopic retrograde cholangiopancreatography (ERCP) is one of the methods for diagnosing and treating fascioliasis. Here we report a case of fascioliasis due to *F. gigantica* that was treated by endoscopic extraction and antiparasitic medication. The causative agent was identified by PCR and DNA sequencing of COI gene.

**CASE REPORT**

A 36-year-old Thai woman, from a rural area of Tak Province, western Thailand, was admitted to Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai. The patient presented with right-upper-quadrant abdominal pain with low-grade fever during the previous 3 months. She had no history of jaundice, pruritus, diarrhea, or weight loss. She had received treatment for dyspepsia, but this had no effect. She had no known underlying diseases and no family history of liver disease or cancer. She had a habit of eating uncooked aquatic vegetables. Over the past 10 years, she complained of intermittent right-upper-quadrant pain. Four months prior to presentation, she underwent an abdominal ultrasound of the upper abdomen, which showed intraluminal echogenicity in the common bile duct without posterior acoustic shadow that was indeterminate in nature. It was initially reported as suggestive of acute calculus cholecystitis.

Laboratory investigations showed hemoglobin level of 12.9 g/dl, a hematocrit of 39.2%, a white blood cell count of 6,600/mm$^3$ with 43.1% neutrophils, 19.6% lymphocytes and 21.8% eosinophils, and a platelet count of 229,000/mm$^3$. Liver and renal function tests were normal. Stool was not examined for parasites.

CT scan of the upper abdomen revealed several subcapsular, wedge-shaped, hypo-enhanced lesions associated with subcapsular retraction and peripheral mild intrahepatic duct dilatation in
Fig 1–Computerized tomography of the upper abdomen showing several subcapsular, wedge shaped, hypo-enhanced lesions associated with subcapsular retraction (a) and peripheral mild intrahepatic duct dilatation in hepatic segments 4a, 5 and 8 (b). Diffuse mild dilatation of the extrahepatic bile duct with mild ductal wall thickening (black arrows in c and d) is seen.

hepatic segments 4a, 5 and 8 (Fig 1). Diffuse mild dilatation of the extrahepatic bile duct with mild ductal wall thickening was also found. From these results in relation to peripheral eosinophilia, chronic eosinophilic liver abscesses and biliary stage of fascioliasis were suspected. The patient underwent ERCP. Mild dilatation of the common bile duct was found without any definite filling defect (Fig 2). A balloon catheter was used to clear the common bile duct revealing a large fluke, which had passed through the papillary opening to the duodenum (Fig 3A).

The fresh fluke (Fig 3B) was compressed, fixed with alcohol-formalin-acetic acid solution and stained with carmine stain (Fig 3C). The body length

Fig 2–Cholangiogram showing mild dilatation of common bile duct (arrow) without any definite filling defect.
Fig 3–Fluke (A, arrow) removed from patient’s common bile duct using a balloon catheter. B. Extracted fresh *Fasciola* sp (37x10x0.5 mm). C. Carmine-stained extracted *Fasciola* sp (bar = 1 cm).
Fig 4 – Maximum likelihood phylogenetic tree of *Fasciola* mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. The phylogenetic tree was constructed using MEGA version 7.0.25 software (Kumar *et al.*, 2016), and genetic distances were estimated using Kimura 2-parameter model. COI sequence of *Paragonimus westermani* (AF219379), a lung fluke, was used as the outgroup. Alignment gaps were treated as partial deletion with 95% site coverage cutoff and bootstrap with 1,000 replications being conducted. Bootstrap values are shown on the nodes of the tree. Scale bar indicates number of substitutions per site. *This study.

was 4.40 cm, body width 1.90 cm, cephalic cone length 0.54 cm, cephalic cone width 0.65 cm, oral sucker 0.16 cm, and ventral sucker or acetabulum 0.19 cm. No intrauterine eggs could be identified in the fluke observed microscopically. The fluke was identified as *Fasciola* sp. The patient was treated with triclabendazole (10 mg/kg).

Identification based on mitochondrial COI DNA sequence was also conducted. Fluke tissue (~10 mg) excised from the posterior region, was homogenized using a plastic pestle in 100 µl of DNAzol® reagent (Invitrogen, Carlsbad, CA), centrifuged at 10,000g for 10 minutes, supernatant mixed with absolute ethanol, centrifuged at 10,000g for 10 minutes, and pellet dissolved in 20 µl of distilled water. DNA concentration was measured using Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) and sample stored at -20°C until used. A 447-bp fragment of COI gene was amplified...
in a 50-µl mixture containing 5 µl of 10X PCR buffer (Invitrogen), 1.5 mM MgCl₂, 200 µM dNTPs, 0.4 U Platinum Taq DNA polymerase (Invitrogen), 100 ng of DNA, 0.5 µM each JB3F forward primer (5′-TTTTTTGGGCATCCTGAGGTT-TAT-3′) and JB4.5R reverse primer (5′-TA-AAGAAAGACATAATGAAAATG-3′). Thermocycling was performed in a Tper personal PCR machine (Biometra, Göttingen, Germany) as follows: 95°C for 2 minutes; 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds; with a final step at 72°C for 2 minutes. Gel-purified amplicon was sequenced in both directions (1st Base, Selangor, Malaysia). A phylogenetic tree was constructed using MEGA software, version 7.0.25 (MEGA, Pennsylvania State University, PA) (1,000 bootstraps) to compare the sequence obtained in this study, Fasciola_TAK (399 bp after trimming) (GenBank accession no. KY963512) with those currently available in GenBank (Fig 4). Fasciola_TAK is located in *F. gigantica* clade, clearly separated from that of *F. hepatica*, with identical sequence to *F. gigantica* (KF687898) collected previously from a patient in Thailand. Fasciola_TAK is also clustered with those of *F. gigantica* (KF687893-7) collected from cattle in Thailand (Wannasan et al, 2014) and *F. gigantica* (GQ121277) from Turkey.

**DISCUSSION**

Clinical diagnosis for fascioliasis is generally based on several criteria: patient’s history of obstructive jaundice, fever and right-upper-quadrant pain; intrahepatic cystic lesions with absolute peripheral blood eosinophilia; and surgical pathology, ultrasonography and CT evidence. However, ultrasonography and CT imaging did not help clarify differential diagnosis, due to technical limitations, which made the bile duct details obtained by these methods inferior to that obtained with ERCP.

For this reason, ERCP, which is capable of showing the presence of fluke in the biliary bile duct, is considered the gold standard for bile duct imaging (Osman *et al*, 1998; Suhocki, 2004). Likewise, ERCP should be the first diagnostic tool of choice for patients in the chronic phase of fascioliasis, even if the diagnosis is initially established by ultrasound or CT imaging (Dowidar *et al*, 1999). ERCP and sphincterotomy were used to remove parasites from the biliary tree by balloon catheter or basket (Condomines *et al*, 1985; Veerappan *et al*, 1991).

Similarly, we successfully used ERCP to remove *F. gigantica* from the common bile duct by balloon catheter. In this case, ERCP proved to be an effective diagnostic and treatment option for a patient with severe abdominal pain and fever that correlated with peripheral eosinophilia, but without jaundice. We endorse ERCP as an effective method for managing fascioliasis.

It is difficult to identify, morphologically, the specific species of fresh worm samples. While carmine staining is a common method for examining the detailed characteristics of flukes, identifying *Fasciola* spp remains difficult, owing to the overlapping morphological characteristics between *F. gigantica* and *F. hepatica* (Lotfy *et al*, 2002; Valero *et al*, 2012). Molecular techniques have been used to solve this problem (Adlard *et al*, 1993; Itagaki and Tsutsumi, 1998; Marcilla *et al*, 2002; Itagaki *et al*, 2005a,b; Ichikawa *et al*, 2010; Rokni *et al*, 2010). Chaichanasak et al (2012) identified 147 *F. gigantica* specimens obtained from cattle in Thailand based on ITS1 and NDI gene sequences. Recently, 15 flukes
from cattle and 7 flukes from 7 clinical cases admitted at Maharaj Nakorn Chiang Mai Hospital were confirmed to be *F. gigantica* by analyses of NDI, COI and ITS2 sequences (Wannasan et al., 2014). In this study, we employed COI gene sequence to identify *F. gigantica* as the infective organism. Although for clinical treatment exact identification of the *Fasciola* fluke may not be necessary, it can help to understand the epidemiology of this parasite.

**ACKNOWLEDGEMENTS**

This study is partially supported by Korea International Cooperation for Infectious Diseases (KOICID).

**REFERENCES**


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