Cystadenocarcinoma of the intrahepatic bile duct in a dog

Ji-Hoon KANG1), Seoung-Yob AHN2), Hun-Young YOON1,3)*

1)Department of Veterinary Surgery, College of Veterinary Medicine, Konkuk University, Seoul, South Korea
2)VIP Animal Medical Center, Seoul, Republic of Korea
3)KU Center for Animal Blood Medical Science, Konkuk University, Seoul, South Korea

ABSTRACT. A 14-year-old spayed female Shih-Tzu was referred to the Veterinary Medical Teaching Hospital of Konkuk University for evaluation of an abdominal mass. In diagnostic imaging, two large cystic masses were identified. The affected liver lobes were surgically resected, and the specimens were submitted for histopathological evaluation and immunohistochemical staining. The two cystic lesions were diagnosed as biliary cystadenocarcinoma (BCAC). Recurrence and regional invasion were identified on ultrasonography 36 days postoperatively. The patient died on postoperative day 271. To the best of our knowledge, previously reported case studies of BCAC in dogs presented limited clinical information. In this report, we present a detailed picture comprising a range of clinical information and histopathological examination of BCAC in a dog.

KEYWORDS: biliary cystadenocarcinoma, cystic tumor, hepatic cyst, hepatic tumor

In dogs and cats, the occurrence of cystadenocarcinoma is very rare. Only a few cases have been reported to date [3, 5, 11, 20, 25]. In most cases, cystadenocarcinomas occur in the ovary or kidney. To the best of our knowledge, only a few cases of biliary cystadenocarcinomas (BCACs) in dogs and cats have been reported [1, 10, 20]. In humans, the incidence of biliary cystic tumors is less than 5% of all solitary cystic lesions in the liver, and the number of reported cases of BCAC is below 200 [6].

The purpose of this report was to provide a detailed description of the clinical picture of BCAC in a dog, since the reported case studies of BCAC in dogs have presented limited clinical information. In this report, we present clinical information on canine BCAC, including the history; results of the physical examination, laboratory investigations, and diagnostic imaging; as well as the surgical management, gross lesions, histopathological results, and prognosis.

A 14-year-old spayed female Shih-Tzu, weighing 4.6 kg, was referred to the Veterinary Medical Teaching Hospital of Konkuk University for evaluation of an abdominal mass. The patient was mildly depressed, and abdominal distension was found on physical examination. There was no remarkable enlargement of the superficial lymph nodes.

A complete blood count revealed leukocytosis (24.33 × 10⁹/L; normal, 6–17 × 10⁹/L) and thrombocytosis (1038 × 10⁹/L; normal, 200–500 × 10⁹/L). In the biochemistry profile, increased alanine aminotransferase (128 U/L; normal, 10–100 U/L), alkaline phosphatase (214 U/L; normal, 23–212 U/L), and gamma-glutamyl transferase (18.7 mg/dL; normal, 0–6 mg/dL) levels; mild hypophosphatemia (3.9 mg/dL; normal, 4.1–5.3 mg/dL); and mildly increased C-reactive protein (41 mg/L; normal, 0–35 mg/L) levels were identified. Electrolyte and coagulation tests were unremarkable. Radiographs revealed a large and oval-shaped mass with soft tissue opacity in the cranioventral abdomen, which had displaced adjacent visceral organs (Fig. 1). Ultrasound (US) revealed two large cystic masses filled with echogenic fluid, which showed moderate vascularity on color Doppler evaluation (Fig. 2). Two masses were located in the left lateral liver lobe and left medial liver lobe. On computed tomography (CT), a well-circumscribed, rounded, hypoattenuating, and cystic mass and a larger well-circumscribed, partially septate (multilocular), round- to oval-shaped, hypoattenuating, cystic mass were identified in the left medial and lateral lobes of the liver, respectively (Fig. 3). The width, length, and height of the first lesion were 42.5 mm, 35.4 mm, and 30.2 mm, respectively, while those of the other lesion were 82.6 mm, 98.6 mm, and 63.0 mm, respectively. There was no evidence of metastasis on radiographs, US, or CT. When synthesizing physical examination, blood laboratory, and diagnostic imaging information, hepatobiliary neoplasia, hepatic abscesses, and hepatic cysts were considered as the differential diagnostic list. Like other neoplasms that occur in the liver, there were no specific clinical features except for multiple cystic structures in the masses. Fine needle aspiration was not performed due to the risk of rupture and needle tract dissemination. After 4 days, surgical resection was performed to diagnose hepatic masses for a treatment plan and to decrease the risk of rupture or clinical signs caused by the space-occupying masses.
The patient was premedicated with cefazoline (22 mg/kg, IV; Cefozol inj., Hankuk Korus Pharm. Co., Ltd., Kangwon-Do, Korea), atropine (0.02 mg/kg, IV; atropine sulfate, JEIL Pharm. Co., Ltd., Daegu, Korea), and butorphanol (0.2 mg/kg, IV; Butorphan inj., Myungmoon Pharm. Co., Ltd., Seoul, Korea). Anesthesia was induced with propofol (4 mg/kg, IV; Anepol inj., Hana Pharm. Co., Ltd., Kyonggi-Do, Korea). General anesthesia was maintained with isoflurane in 100% oxygen. The patient was positioned in dorsal recumbency, and the entire ventral abdomen and caudal thorax were clipped and prepared for a cranioventral midline incision. A ventral abdominal incision was performed from the xiphoid process to near the pubis. After resecting the falciform ligament, the entire abdomen was explored for metastases.

After ventral midline incision, the mass in the left lateral liver lobes, which adhered minimally to the greater omentum, was identified (Fig. 4A). For liver lobectomy, the left lateral liver lobes were dissected from the surrounding tissues. The masses were resected using a thoracoabdominal stapler and full-circumferential ligation. The mass in the left medial liver lobe was identified after the left lateral liver lobe was resected (Fig. 4B). The left medial liver lobe was resected during the same procedure. After the liver lobes containing the masses had been resected, the resection sites were investigated for hemorrhage and other problems. No other metastatic or invasive lesions were found intraoperatively. The abdominal wall was closed as per routine.

The masses had cystic structures filled with mucinous, cloudy, white-colored fluid and ivory-colored inner walls (Fig. 5). The mucinous fluid in the masses was positive on periodic acid–Schiff (PAS) staining (Fig. 6). The margins of the masses were uncertain, and the cell sizes varied, with mitoses observed on histopathologic examination of the inner walls of the cystic structures. Bile duct structures were also identified with abnormal lining cells (Fig. 7). On immunohistochemistry (IHC), both masses were cytokeratin antibody (AE1/AE3)-positive and vimentin stain-negative, indicating an epithelial origin (Fig. 8). Because the two masses had distinctive characteristics, such as multilocular lesions with fluid contained mucin, and the results of histopathology and IHC staining indicated bile duct adenocarcinoma, the patient was diagnosed with an intrahepatic bile duct cystadenocarcinoma. Although there

Fig. 1. On radiographs, a large and oval-shaped mass was identified in the cranioventral abdomen (*). This mass had soft tissue opacity and displaced adjacent visceral organs.

Fig. 2. On ultrasonography, two large cystic masses were identified in the left lateral liver lobe (M1) (A and B) and left medial liver lobe (M2) (C). In these masses, echogenic fluid was filled with a multilocular structure. Echogenicity of the liver parenchyma adjacent to mass was increased (yellow arrow). Mass in the left medial liver lobe was adjacent to the gallbladder (GB). On Doppler evaluation, two masses showed moderate vascularity.
were no other masses in US, CT, and exploratory laparotomy, it was not possible to distinguish which of the two masses was the original neoplasm.

Five days postoperatively, the patient was discharged with no clinical signs. However, 36 days postoperatively, the patient was rechecked for metastasis. Although there was no evidence of metastasis on radiographs, US revealed a small cyst measuring 5.1 mm in width and 4.2 mm in length in the papillary process of the caudate liver lobe. A cyst was also identified in the head of the spleen.
These two lesions were considered to be regional recurrences. Because the owner refused both surgery and chemotherapy, the patient was regularly rechecked for metastasis and given supportive therapy. On postoperative day 154, thoracic metastasis was suspected on radiography, and regional recurrence to the remaining liver lobe was identified on US. On postoperative day 200, the patient had anorexia, vomiting, diarrhea, and seizure. The patient died on postoperative day 271.

According to the World Health Organization classification of tumors, BCAC is a unilocular or multilocular glandular tumor [24]. Anatomically, the biliary system can be divided into three segments: the intrahepatic bile duct, extrahepatic bile duct, and gallbladder [17, 20]. Carcinomas of the biliary system have been reported in all three segments in dogs and cats, and the intrahepatic bile duct is a common site for carcinomas in all animals except swine [17]. Histologically, malignant tumors that occur in the bile duct are categorized as tubular carcinomas and BCACs [20]. The cysts of BCACs are lined with eosinophilic cuboidal or columnar cells. If the cysts communicate with the biliary system, they may contain bile, and the color of the cystic fluid may be yellowish. However,
if the cysts do not contain bile, but only serosanguinous fluid, the color of the cystic fluid may be cloudy white. Tumors occurring in the biliary system are common in humans, although biliary cystic tumors usually do not communicate with the biliary system [6]. The BCAC in our case was a multilocular glandular tumor with ivory-colored inner walls and white-colored cystic fluid, indicating that the BCAC did not communicate with the biliary system.

In dogs, most cases of cystadenocarcinoma involve the ovary or kidney [3, 5, 11, 25]. In cases of ovarian cystadenocarcinomas, tumors may secrete hormones, such as estrogen [25]. Hormone-secreting ovarian papillary cystadenocarcinomas can cause the vaginal fold to prolapse and pyometra. If regional metastasis occurs, surrounding organs, such as the small intestine, may be involved, and clinical signs associated with the metastatic organ may be present [11]. In cases of renal cystadenocarcinoma, cystic changes of the kidney and nodular dermofibrosis may occur, and clinical signs and laboratory findings associated with kidney dysfunction may be observed [3, 5]. German shepherd dogs are predisposed to renal cystadenocarcinomas, which may be attributed to a genetic mutation [14]. Since most cases of renal cystadenocarcinomas occur with nodular dermofibrosis, renal cystadenocarcinomas are referred to as renal cystadenocarcinomas and nodular dermofibrosis (RCND) [3, 5]. The current patient had mildly elevated liver enzymes but no other skin lesions that suggested RCND. In our case, only abdominal distension was found on physical examination, as expected with liver tumors. No other remarkable clinical signs were identified. There was no cystic lesion on US and CT evaluation, except for the liver masses.

The causes of cystic lesions in the liver can be congenital, traumatic, infectious, parasitic, or neoplastic [19]. Although cystic lesions can replace the liver parenchyma, compress it mechanically, or become secondarily infected, most cystic lesions are usually detected incidentally [19]. Neoplastic cysts can be differentiated from non-neoplastic cysts, such as hepatic hematomas or simple hepatic cysts, using color Doppler evaluation [16, 19]. In human studies, US, CT, and magnetic resonance imaging (MRI) have been used to diagnose BCAC [16]. CT and MRI can be used to differentiate between benign and malignant tumors [4, 13]. On CT evaluation, features related to malignant tumors are post-contrast enhancement patterns in the delayed phase and the maximal transverse diameter of the lesion [13]. On MRI evaluation, heterogeneous enhancement of malignant tumors can be identified in T1-weighted post-contrast and T2-weighted images [15]. The overall sensitivity and specificity of MRI for differentiating malignant from benign lesions are 100% and 90%, respectively, using the inherent high soft-tissue contrast [4]. To the best of our knowledge, however, CT and MRI studies on biliary cystadenomas (BCAs) or BCACs in dogs and cats have been limited. Furthermore, our patient was not evaluated using MRI. Cystic lesions of the liver in our case had not been diagnosed preoperatively for this reason. Furthermore, CT fares better than MRI for distinguishing BCACs from BCAs, and the findings obtained with MRI were not different from those obtained with CT in some human studies [2].

The various types of tumors occurring in the liver include hepatocellular, bile duct, and neuroendocrine tumors (carcinoid), along with sarcomas and metastatic tumors [19]. BCAC is classified as a variant of cholangiocellular carcinoma [17]. To diagnose cholangiocellular carcinoma, IHC can be a useful diagnostic tool [18, 22]. Tumors with cytokeratin-positive cells are considered to be tumors of epithelial origin, because these filaments act as a cytoskeletal structure for epithelial cells. This marker may be superior for micro-metastases to regional lymph nodes in patients with BCACs [18]. Vimentin is another intermediate filament of normal mesenchymal cells [22]. Although vimentin staining can be used as a marker of epithelial–mesenchymal transition in some tumors, it can also serve as a diagnostic tool for tumors of mesenchymal origin. In one study of IHC in human BCAC, the lining epithelial cells were positive for cytokeratin (11/11) and almost negative for vimentin (2/11) [7]. Additionally, specific markers, such as carcinoembryonic antigen or carbohydrate antigen 19–9, can be used for differential diagnosis in humans [23, 26]. If a hepatic mass was diagnosed in cholangiocellular carcinoma, it is not difficult to distinguish BCAC from cholangiocellular carcinoma. BCAC has specific morphological characteristics. Although all BCACs do not have singular morphological characteristics, internal septation and nodularity are present in BCACs. Additionally, each cystic structure is filled with fluid containing mucin. On histopathology, the internal walls of BCAC consist of a dense connective tissue lining, with single or multiple layers of mucin-secreting cells. In cholangiocellular carcinoma, however, the affected liver lobe is atrophied because the portal vein is obstructed by cholangiocellular carcinoma in most cases. The texture of cholangiocellular carcinoma is also an important gross pathological feature [17]. Cholangiocellular carcinomas are firm in most cases, due to abundant connective tissue, while hepatocellular carcinomas are typically soft and friable [17]. In our case, hepatic masses had multilocular structures on US and CT. The texture of the masses was not firm, and multilocular structures were identified. Fluid collected from the hepatic masses intraoperatively was positive on PAS stain, which implies that mucin is present in the fluid. These findings were not in accordance with cholangiocellular carcinoma. On histopathological and IHC studies, structures of bile ducts with malignancy and epithelial origin were also identified. Cystic structures were lined with single layer of columnar cells. Therefore, the hepatic masses in our case were diagnosed as BCACs.

In humans, if there is no evidence of metastasis, the prognosis of BCAC is good, and the recurrence rates are low (approximately 5–10%) [2, 12, 21, 22, 25]. However, BCACs in dogs and cats have a high rate of metastasis [20]. In that study, all cases (12/12) were reported to have metastases. The sites of metastases were the lymph nodes (67%); lungs (54%); peritoneal implants (46%); spleen and adrenal glands (8%); and heart, pancreas, kidney, and spinal column (4%). In our case, two large cystic lesions were identified, and no metastatic lesions were present preoperatively. However, recurrence in the papillary process of the caudate liver lobe was identified 36 days postoperatively, indicating that microinvasion into the local liver lobe had already occurred when the surgery was performed.

Chemotherapy was performed in one case of BCAC [10]. In this study, a cat with BCA that had undergone malignant transformation underwent an incisional biopsy and chemotherapy. Doxorubicin was chosen initially, although this was changed to carboplatin 7 weeks postoperatively. The cat’s appetite was poor, and peritoneal effusion recurred rapidly. He was euthanized on postoperative day 54. However, successful treatment with chemotherapy has been reported in humans [9]. In one case, a 78-year-old woman with BCAC was treated with hepatic arterial infusion chemotherapy with cisplatin. After 8 months of chemotherapy, the mass had decreased from...
BILIARY CYSTADENOCARCINOMA IN A DOG

12 cm to 8 cm in diameter [8]. In another case, a 60-year-old woman with BCAC underwent left lobectomy. The BCAC recurred 41 months postoperatively, and she was treated with chemotherapy. Systemic administration of 5-fluorouracil and cisplatin considerably reduced the metastatic tumor and ascites. The patient’s general condition improved, and chemotherapy was continued [9]. In our case, chemotherapy was not used because reported studies about chemotherapy for cystadenocarcinomas in dogs were limited, and the owner refused chemotherapy.

BCAC is a very rare tumor in dogs and cats, and its prognosis is poor, unlike that in humans. Furthermore, diagnosis of BCAC is difficult because most cystic lesions in the liver appear similar on diagnostic imaging. The treatment options for BCAC are surgery and chemotherapy. However, metastasis is common when surgery is chosen, and the effect of chemotherapy has not yet been proven and requires further study.

CONFLICT OF INTEREST. The authors declare that there were no conflicts of interest

REFERENCES

1. Argenta FF, Mello LS, Caprioli RA, Pavarini SP, Driemeier D, Sonne L. 2020. Pathological and immunohistochemical aspects of primary hepatobiliary neoplasms in cats. *Pesqui Vet Bras* 40: 46–54. [CrossRef]
2. Choi HK, Lee JK, Lee KH, Lee KT, Rhee JC, Kim KH, Jang KT, Kim SH, Park Y. 2010. Differential diagnosis for intrahepatic biliary cystadenoma and hepatic simple cyst: significance of cystic fluid analysis and radiologic findings. *J Clin Gastroenterol* 44: 289–293. [Medline] [CrossRef]
3. Ciccarelli S, Di Bello A, Valastro C, Leo C, Lenoci D, Rana E, Franchini D. 2019. Unilateral renal cystadenocarcinoma and nodular dermofibrosis in a mixed-breed dog carrying a FLCN gene mutation. *Vet Dermatol* 30: 174–154. [Medline] [CrossRef]
4. Clifford CA, Pretorius ES, Weisse C, Sorenko KU, Drobatz KJ, Siegelman ES, Solomon JA. 2004. Magnetic resonance imaging of focal splenic and hepatic lesions in the dog. *J Vet Intern Med* 18: 330–338. [Medline] [CrossRef]
5. Conrado ALV, Iunes RS, Balduinio ALL, Santanna MCFB, da Silva JRMC. 2020. Serum symmetric dimethylarginine levels in a half-breed German shepherd dog with renal cystadenocarcinoma and nodular dermofibrosis. *Comp Clin Pathol* 29: 905–909. [CrossRef]
6. Del Poggio P, Buonocore M. 2008. Cystic tumors of the liver: a practical approach. *World J Gastroenterol* 14: 3616–3620. [Medline] [CrossRef]
7. Devaney K, Goodman ZD, Ishak KG. 1994. Hepatobiliary cystadenoma and cystadenocarcinoma. A light microscopic and immunohistochemical study of 70 patients. *Am J Surg Pathol* 18: 1078–1091. [Medline] [CrossRef]
8. Hanazaki K, Yoshizawa K, Mori H. 1999. Hepatic arterial infusion chemotherapy of cisplatin for biliary cystadenocarcinoma. *Hepatogastroenterology* 46: 462–464. [Medline]
9. Iyama S, Takahashi N, Shintani N, Fujikawa K, Okhubo S, Sato Y, Sato T, Ohnuma K, Niitsu Y. 2006. [A case of recurrent biliary cystadenocarcinoma successfully treated with 5FU/CDDP systemic chemotherapy]. *Nihon Shokakibyo Gakkai Zasshi* 103: 1163–1168 (in Japanese). [Medline]
10. Jacobs TM, Snyder PW. 2007. Mucinous cholangiocarcinoma in a cat. *J Am Anim Hosp Assoc* 43: 168–172. [Medline] [CrossRef]
11. Khaki F, Javanbakht J, Sharifzad S, Ghargozelou MJ, Khadivar F, Manesh JYY, Hosseini SH, Anissian A, Touni SR, Gilvari A, Abdi FS. 2014. Metastatic ovarian papillary cystadenocarcinoma to the small intestine serous surface: report of a case of high-grade histopathologic malignancy. *J Ovarian Res* 7: 33 (in Korea). [Medline] [CrossRef]
12. Kim HG. 2006. Biliary cystadenoma: biliary cystadenoma and biliary cystadenocarcinoma. *Korean J Gastroenterol* 47: 5–14 (in Korea). [Medline]
13. Leela-Arpong R, Ohta H, Shimbo G, Hanazono K, Osuga T, Morishita K, Sasaki N, Takiguchi M. 2019. Computed tomographic features for differentiating benign from malignant liver lesions in dogs. *J Vet Med Sci* 81: 1697–1704. [Medline] [CrossRef]
14. Lingaas F, Comstock KE, Kirkness EF, Sørensen A, Aarskaug T, Hitte C, Nickerson ML, Moe L, Schmidt LS, Thomas R, Breen M, Galibert F, Zbar B, Ostrander EA. 2003. A mutation in the canine BHD gene is associated with hereditary multifocal renal cystadenocarcinoma and nodular dermofibrosis in the German Shepherd dog. *Hum Mol Genet* 12: 3043–3053. [Medline] [CrossRef]
15. Mai W., editor. 2018. Diagnostic MRI in Dogs and Cats, 1st ed., CRC Press, Boca Raton.
16. Mavilia MG, Pakala T, Molina M, Wu GY. 2018. Differentiating cystic liver lesions: a review of imaging modalities, diagnosis and management. *J Clin Transl Hepatol* 6: 208–216. [Medline] [CrossRef]
17. Meuten DJ. 2020. Tumors in Domestic Animals, 5th ed., John Wiley & Sons, Oxford.
18. Natarajan S, Xu F, Gilchrist K, Weber SM. 2005. Cytokeratin is a superior marker for detection of micrometastatic biliary tract carcinoma. *J Surg Res* 125: 9–15. [Medline] [CrossRef]
19. Nyland TG, Mattoon JS. 2002. Small Animal Diagnostic Ultrasound, Elsevier Health Sciences, Philadelphia.
20. Patnaik AK, Hurvitz AI, Lieberman PH, Johnson GF. 1981. Canine bile duct carcinoma. *Vet Pathol* 18: 439–444. [Medline] [CrossRef]
21. Ramia JM, de la Plaza R, Pérez Mies B, Arteaga V, Garcia-Parreño J. 2015. Biliary cystadenocarcinoma. *Cir Esp* 93: e53–e55 (in Spanish). [Medline] [CrossRef]
22. Satelli A, Li S. 2011. Vimentin in cancer and its potential as a molecular target for cancer therapy. *Cell Mol Life Sci* 68: 3033–3046. [Medline] [CrossRef]
23. Soares KC, Armaoutakis DJ, Kamel I, Anders R, Adams RB, Bauer TW, Pawlik TM. 2014. Cystic neoplasms of the liver: biliary cystadenoma and cystadenocarcinoma. *J Am Coll Surg* 218: 119–128. [Medline] [CrossRef]
24. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, Chirieac LR, Dacic S, Hirsch FR, Ishikawa Y, Kerr KM, Noguchi M, Pelosi G, Powell CA, Tsao MS, Wistuba I. WHO Panel. 2015. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. *J Thorac Oncol* 10: 1243–1260. [Medline] [CrossRef]
25. Zen Y, Pedica F, Patcha VR, Capelli P, Zamboni G, Casaril A, Quaglia A, Nakanuma Y, Heaton N, Portmann B. 2011. Mucinous cystic neoplasms of the liver: a clinicopathological study and comparison with intraductal papillary neoplasms of the bile duct. *Mod Pathol* 24: 1079–1089. [Medline] [CrossRef]