CHOLANGIOCARCINOMA OF INTRAHEPATIC BILE DUCTS WITH DISSEMINATED METASTASES IN AN AFRICAN LION (PANTHERA LEO)

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CHOLANGIOCARCINOMA OF INTRAHEPATIC BILE DUCTS WITH DISSEMINATED METASTASES IN AN AFRICAN LION (PANTHERA LEO)

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Abstract: A cholangiocarcinoma is reported in an 18-yr-old, female African lion (Panthera leo). The primary tumor consisted of multifocal to coalescing, hepatic, white–yellow masses distributed throughout the liver lobes. Metastases were present in regional lymph nodes, peritoneal surface, and lungs. Histologically, the tumor was characterized by a tubular pattern with alcian- and periodic acid–Schiff-positive secretory material in cystic spaces. The neoplastic cells were positive to broad-spectrum cytokeratins. Histochemical and immunohistochemical stains were consistent with bile duct carcinoma. Biliary tumors arising from the gallbladder have been reported in lions. However, to the authors’ knowledge, this is the first case of intrahepatic bile duct carcinoma reported in an African lion.

Key words: African lion, bile ducts, cholangiocarcinoma, liver, Panthera leo, tumor.

BRIEF COMMUNICATION

Bile duct tumors are primary hepatic neoplasms reported in both domestic and wild animals, including wild felids such as margay (Felis wiedii), black leopard (Panthera pardus), and lion (Panthera leo). They can arise from intrahepatic and extrahepatic ducts as well as from the gallbladder. Tumors in the African lion are rarely reported, mostly as single case report or small reviews. Only two cases of primary biliary adenocarcinomas, arising from the gallbladder, have been described to date; an additional case of biliary cystadenoma described as an incidental finding at necropsy is reported, and recently a lesion described as peribiliary cysts has been described.

An 18-yr-old, female African lion, born in captivity, was presented with a several-week history of progressive worsening of body condition, anorexia, and depression associated, in the last stages, with severe abdominal swelling secondary to peritoneal effusion. Abdominal fluid collected by abdominocentesis was blood-tinged and contained numerous variably sized clusters of closely packed, round to oval cells, characterized by a high nuclear-to-cytoplasmic ratio, small amounts of basophilic cytoplasm, round nuclei with prominent nucleoli, and marked anisokaryosis; these findings were considered consistent with a malignant epithelial neoplasm. As a result of her poor body condition and prognosis the lion was euthanized, and a full necropsy was performed soon after death.

Postmortem examination showed a severe abdominal effusion of about 2 L of turbid, reddish fluid; parietal and visceral peritoneum had multiple, disseminated, firm, white–yellow nodules, ranging from 3 to 10 mm in diameter (Fig. 1). The liver was enlarged, with dozens of multifocal to coalescing, nonencapsulated, welldemarcated, white to yellow, firm nodules. The largest nodule was 8 cm in diameter and was located in the upper caudate lobe, just above the hilus, with many satellite nodules, ranging from 0.5 to 5 cm in diameter, scattered throughout the hepatic lobes (Fig. 2). On cut surface the nodules showed an infiltrative growth pattern, with frequent central necrosis or large cystic cavities containing gray–brown, viscous material. The hepatic lymph nodes were enlarged. No masses were observed in the gallbladder or in the other abdominal organs. Both kidneys were small and firm with an irregular surface, consistent with severe chronic renal disease. Small (0.2–0.5-cm) nodules were scattered in the lung lobes. The other organs were unremarkable.

Samples from the nodules were fixed in 10% neutral buffered formalin, routinely processed, and stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), alcin blue–PAS (pH 2.5), and Warthin–Starry stain. Immunohistochemistry was performed using antibodies against a broad-spectrum cytokeratin (CK) pool (1:250 dilution; clone MNF 116, DAKO Corporation, Carpinteria, California 93013, USA), a high-mo-
molecular-weight (HMW) cytokeratin (1:50 dilution; clone 34βE12, DAKO Corporation), and vimentin (1:200 dilution; clone V9, DAKO Corporation). Briefly, slides were rehydrated, antigen retrieval was obtained by use of proteinase K for CK-MNF, and microwave treatment ensued for 20 min at the highest power in a preheated Tris–ethylenediamine tetraacetic acid (EDTA) buffer solution (10 mM/L Tris base, 1 mM/L EDTA, pH 6.0) for vimentin; no retrieval was applied for CK-HWM. A streptavidin–biotin protocol was applied according to the manufacturer’s instructions. 3-Amino-9-ethylcarbazole was used as chromogen (AEC + Substrate-Chromogen Ready-to-use, DakoCytomation), and Carazzi’s hematoxylin was used as the counterstain.

Histologically the tumor was composed by tubules and cords of neoplastic cells separated by a variable amount of fibrous stroma (desmoplasia) and multifocal large cysts containing necrotic debris and eosinophilic amorphous material, with intraluminal papillary projections (Fig. 3). In some areas, the tumor showed a more solid growth pattern, with marked cellular atypia. Tubules were composed of cuboidal to columnar cells with scant lightly basophilic apical cytoplasm containing PAS-positive small droplets, basal large hypochromic nuclei, and single prominent nucleoli. A delicate PAS-positive border was also observed in the apical membrane of neoplastic cells. Occasionally, the lumen of neoplastic acini contained basophilic amorphous material intensely stained with PAS and alcian blue. The mitotic index measured seven mitoses per 10 high-powered fields, higher in more undifferentiated areas. In the lymph node metastases, the tumor showed a more undifferentiated morphology. In the lungs, multiple scattered nodules with necrotic centers and a peripheral rim of neoplastic tissue similar to the primary tumor were present. Gross, histologic, and histochemical findings were consistent with cholangiocellular carcinoma with peritoneal carcinomatosis and nodal and pulmonary metastases.

Immunohistochemistry revealed a focally intense cytokeratin immunoreactivity both in the primary and in the metastatic nodules, whereas vimentin was negative in all of the samples. These results supported the diagnosis of cholangiocarc-
cinoma since neoplastic biliary epithelium, but neither hepatocytes nor hepatocellular tumors, reacts to wide-spectrum cytokeratins and HMW cytokeratins. In our case, as in those reported by Sakai et al., neoplastic cells were positive for cytokeratins (MNF 116-34bE12 and AE1/AE3-CK7, respectively) and negative for vimentin.

Differential diagnoses for cholangiocarcinoma include hepatocellular carcinoma or secondary epithelial tumors from other abdominal organs (pancreas, intestine, or ovary).

In this present study, hepatocellular carcinoma was ruled out based on histomorphology (tubular and acinar pattern, desmoplasia, secretory cysts, high mitotic index), histochemistry (presence of small PAS- and alcian-positive droplets in the cytoplasm), and immunohistochemical features (immunoreactivity to cytokeratins). No other masses were detected at necropsy, allowing a secondary tumor to be excluded.

Biliary tumors can arise either from bile ducts, both intrahepatic and extrahepatic, and gallbladder and can present as single or multiple masses as a result of a multicentric origin or intrahepatic metastases. Hematogeneous spread to lungs and transcoelomic metastases to peritoneal serosal surfaces are common.

Clinically, this current case is quite similar to previously reported biliary carcinomas in African lions, but the necropsy findings differ, as those cases had a severe thickening of the gallbladder, with liver nodules mainly in the perihilar area, indicating a direct extension from an infiltrative primary gallbladder tumor. In this current case, the gallbladder was grossly and microscopically normal, and nodules were homogeneously distributed throughout the hepatic lobes; this pattern of distribution is consistent with an intrahepatic bile duct origin and possible intrahepatic metastases rather than a multicentric origin, as indicated by the presence of the largest nodule with high mitotic index, histochemistry (presence of small PAS- and alcian-positive droplets in the cytoplasm), and immunohistochemical features (immunoreactivity to cytokeratins). No other masses were detected at necropsy, allowing a secondary tumor to be excluded.

Primary hepatic tumors are uncommon in domestic animals; however, cholangiocarcinoma is the more common primary hepatic tumor of domestic cats. The etiology of bile duct tumors is unknown, although some chemical and biological agents have been proposed. Among the latter, liver fluke infestations have been related to spontaneous bile duct tumors both in dogs and cats as well as in human beings. Furthermore, cholangiocarcinoma has been experimentally induced in Syrian hamsters by infestation with Opisthorchis viverrini; the carcinogenetic mechanisms of fluke infestation remain unclear, but chronic irritation, nitric oxide formation, intrinsic nitrosation, and activation of drug-metabolizing enzymes have been proposed. These parasites have an indirect life cycle, including a secondary intermediate host generally represented by fishes. In this case, the lion was fed with fresh meat, and fish was not included in the diet; therefore, an association with parasitism is unlikely.

Recently, bacteria of the genus Helicobacter (Helicobacter pylori, Helicobacter hepaticus, and Helicobacter bilis) have been detected by polymerase chain reaction (PCR) in the liver of humans affected by neoplastic biliary tree lesions, but their role in the development of neoplasia needs to be further investigated. In animals, H. hepaticus has been isolated from the liver of mice affected by chronic active hepatitis and associated hepatic tumors, and spiral-shaped argirophilic bacteria consistent with Helicobacter spp. and Helicobacter cholecytus have been demonstrated by PCR and cultured from liver of ferrets with chronic biliary disease and biliary tumors. In this lion, no spiral-shaped bacteria were identified in the tumor and in normal biliary ducts by silver stains.

Environmental chemicals, mycotoxins, and chronic irritation are also considered as potential carcinogenic factors. However, no predisposing factors were identified in this present case. Interestingly, an hepatic cystadenoma was incidentally found at necropsy in another lion coming from the same group, but no correlation between the two cases could be verified.

The overall incidence of tumors in African lion is unknown; however, based on the current literature, bile duct neoplasms seem to be among the most commonly reported tumors in this species and in other wild felids (similar to the high prevalence of bile duct tumors among primary hepatic neoplasms in the domestic cat). These tumors can be associated with nonspecific clinical signs, making ante-mortem diagnosis difficult, and can remain undetected until a terminal stage. In this case, an ante-mortem diagnosis was suspected on the basis of a cytologic examination of the peritoneal effusion; this is a rapid and inexpensive method but it has a low sensitivity for
the detection of hepatic tumors, as they frequently grow into hepatic parenchyma and exfoliate few cells into the peritoneal cavity, unless a massive peritoneal seeding of neoplastic cells develops in the later stages of the disease. Hepatic bile duct tumors might be considered an underestimated tumor in large felids, and their pathogenesis will need further investigation.

**LITERATURE CITED**


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ERRATUM

The authors of the manuscript entitled “Cholangiocarcinoma of Intrahepatic Bile Ducts with Disseminated Metastases in an African Lion (Panthera leo) that was published in 44: 509–512 mistakenly transposed the first and last names of some of the authors in the manuscript as printed as Elvio Lepri, Monica Sforna, Brachelente Chiara, and Vitellozzi Giovanni. The correct author listing should be Elvio Lepri, Monica Sforna, Chiara Brachelente, and Giovanni Vitellozzi.