

Histopathological and immunohistochemical diagnosis of cholangiocellular carcinoma in a Pomeranian dog

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Abstract

A 15 year old Pomeranian breed dog was presented for post mortem examination at Regional Animal Disease Diagnostic Laboratory Visakhapatnam, died with a history of inappetence, vomiting and weight loss. During necropsy examination, ascites, diffuse greyish-white small patches and few nodules with different sizes on the liver surface and diffuse areas of consolidation on the lungs were observed. Histologically, there were tubular structures lined with cuboidal or columnar cells, with diffuse fibrous stroma separating the glandular structures in the liver. Metastatic foci of neoplastic proliferative epithelial cells along the alveolar septum observed in lungs. Cytokeratin 7 expressed diffusely in neoplastic cells of the liver and lung. Based on gross, histopathological and immunohistochemical findings, the present case was confirmed as metastatic cholangiocellular carcinoma.

Keywords: Cholangiocellular carcinoma, Immunohistochemistry, Metastasis

Highlights

- Microscopic examination confirmed cholangiocellular carcinoma.
- Immunohistochemical examination aided in differentiating cholangiocellular carcinoma from hepatic carcinoma.
- Histopathological and immunohistochemical features confirmed the case as cholangiocellular carcinoma.

In dogs, cholangiocellular carcinoma is the second most common liver tumour, accounting for 22% to 41% of all malignant liver tumours. Cholangiocellular carcinoma is aggressive, and metastasis is common (Liptak, 2013). Cholangiocellular carcinomas comprise less than 1% of all neoplasms found in dogs and cats; the incidence of cholangiocellular carcinoma in dogs has been estimated to be 0.36% of all neoplasms (Strafuss, 1976). Cholangiocellular carcinomas have a highly invasive pattern of growth and metastasize frequently in all species. The rate of extrahepatic metastasis in dogs has been reported to be from 60% to 88% (Strafuss, 1976; Patnaik *et al.*, 1981; Trigo *et al.*, 1982). The present report described the histopathological and immunohistochemical findings of metastatic intra-hepatic cholangiocellular carcinoma in Pomeranian dog.

A 15 year old Pomeranian breed dog died with a history of inappetence and weight loss. Detailed post mortem examination was conducted at the Regional Animal Disease Diagnostic Laboratory, Visakhapatnam. At necropsy, ascites, diffuse greyish-white small patches and few nodules with different sizes and cystic space

with gelatinous material were observed on the liver surface (Fig. 1), and diffuse areas of consolidation on the lungs were also observed. Cystic spaces containing mucinous to gelatinous material were gross pathological features more characteristic of cholangiocarcinomas that would help distinguish them from carcinoids, since the lesion was not typical of carcinoids (Meuten, 2016). Representative tissue samples from the liver and lung were collected into 10% neutral buffered formalin for histopathological and immunohistochemical studies. The tissue samples were processed by paraffin-embedding technique. Sections of 4 µm were obtained and stained with routine haematoxylin and eosin staining method. Immunohistochemical staining was performed on liver and lung sections with mouse monoclonal primary antibody to Cytokeratin 7 using Super-sensitive polymer-HRP detection kit (Bio Genex). Tissue sections with 4µ thickness were deparaffinised, rehydrated and transferred to citrate buffer placed in a microwave oven (800 W) for 30 min for antigen retrieval and incubated for 10 min in Peroxide block TM and Power block TM. Primary antibody cytokeratin applied

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Indian Journal of Animal Health, December, 2023
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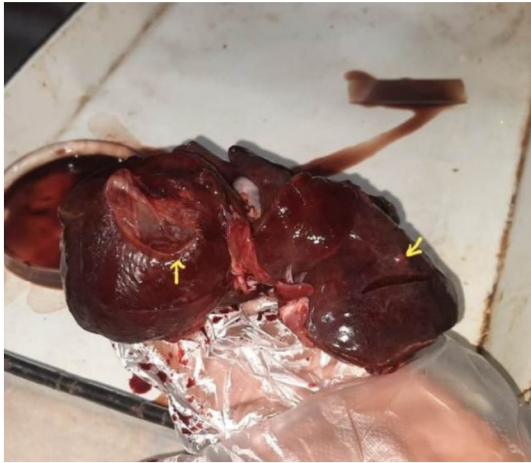


Fig. 1. Liver: Nodules with different sizes and cystic space with gelatinous material

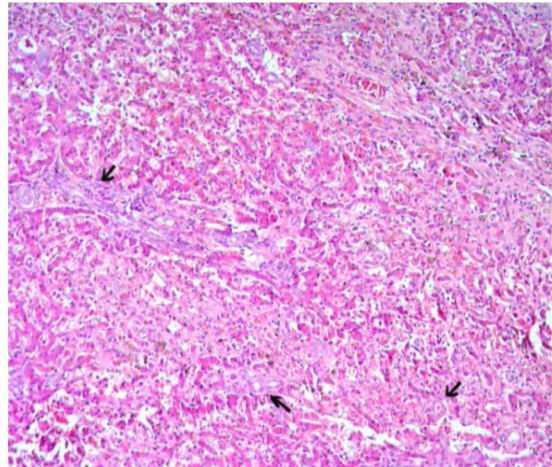


Fig. 2. Liver: Stromal invasion of cuboidal cells forming cords, irregular glandular structures (arrow), H&E, 100X

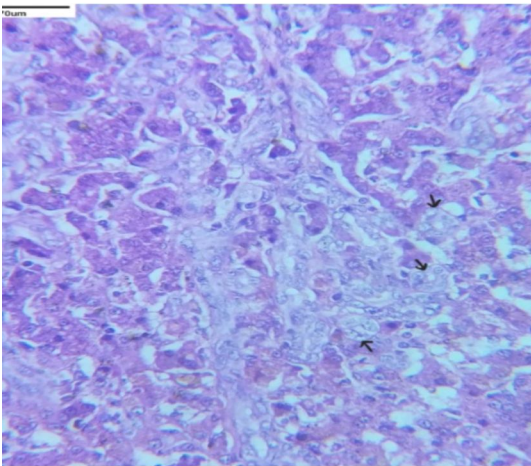


Fig. 3. Liver: Proliferation of epithelial cells arranged in ductal and acinar pattern, H&E, 400X

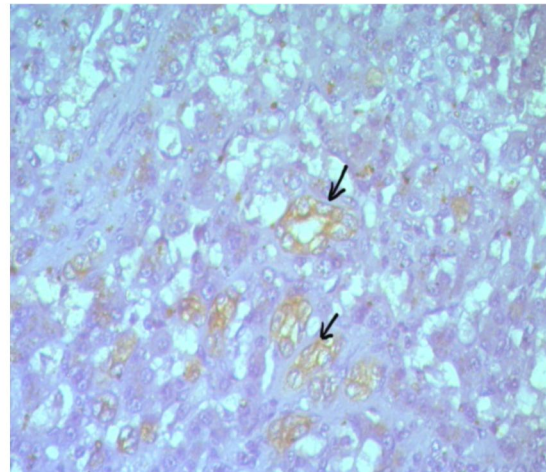


Fig. 4. Liver: Diffuse cytoplasmic staining of epithelial cells arranged in ductal and acinar pattern for cytokeratin, 400X

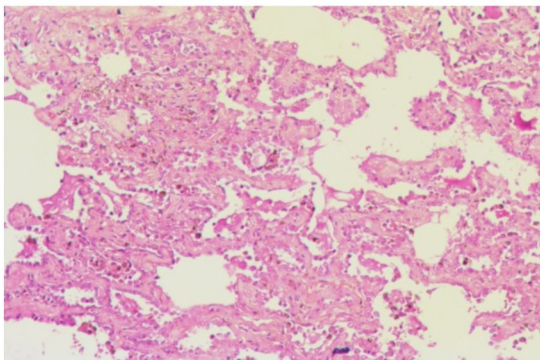


Fig. 5. Lung: Proliferation of epithelial cells along the alveolar septum, H&E, 400X

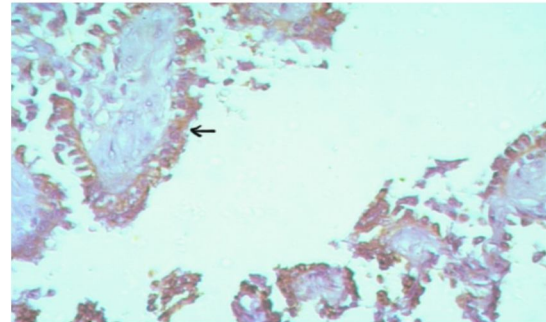


Fig. 6. Lung: Diffuse cytoplasmic staining of epithelial cells along the alveolar septum for cytokeratin, 400X

to the sections and incubated for 60 min at room temperature. The sections were rinsed in TBS, and Super enhancer TM was added to slides incubated for 20 min. Then, the sections were rinsed in TBS buffer and were covered with Poly-HRP reagent for 10 min. Sections were rinsed in TBS buffer. The sections were then incubated with solution prepared by adding one drop of liquid DAB chromogen mixed with 1 mL of buffer for 10 min. Sections were rinsed with TBS, washed with distilled water, counter stained with Harris Haematoxylin and mounted with DPX mountant.

Microscopically, in the liver, neoplastic cells were found to be grown along the surface of the intrahepatic bile duct and showed stromal invasion of cuboidal cells forming cords, irregular glandular structures, numerous mitotic figures along with degenerative changes in hepatocyte were observed (Fig. 2&3). Acinar arrangement of neoplastic cells with intraluminal mucin and fibrous tissues in the surrounding liver parenchyma was observed. Atypical cells were observed along the alveolar wall in few pulmonary areas. Carcinoid cells proliferated along the alveolar septum (Fig. 5). The tumor showed stromal invasion forming irregular tubular structures. Immunohistochemical examination revealed that the tumor cells of the liver and lungs were positive

for CK 7 (Fig. 4 and Fig. 6). Cholangiocellular carcinomas were to be distinguished from metastatic adenocarcinomas to the liver. Neoplastic biliary epithelial cells, but not hepatocytes, could be IHC stained with broadly reactive antibody mixtures that would recognize high and low molecular weight cytokeratins (Patnaik, 1992), although the use of the more specific cytokeratin 7 was considered more reliable (Ramos Vara *et al.*, 2001). Aslan *et al.* (2014) described cholangiocarcinoma of intrahepatic bile ducts with disseminated metastases in a Siamese cat with similar gross, histopathological and immunohistochemical findings.

In conclusion, based on the pathological features observed in the lung tumor that was closely related to the features observed in the liver, the present case was confirmed as metastatic cholangiocellular carcinoma.

Conflict of interest: There is no conflict of interest regarding this work.

Author's contribution: RMK, LR, PSK: Conception and design; MH, GS: Collection of data and sample; BBM, KNB: Processing of samples and interpretation of the results.

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