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Case Report

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A clear cell hepatocellular carcinoma in an obese dog with hyperlipidemia: a case report

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An 11-year-old obese dog was referred for a liver mass. Cytologic examination revealed vacuolated hepatocytes with mild pleomorphism. A partial liver lobectomy was performed. On histopathologic examination, the mass was diagnosed as hepatocellular carcinoma composed of hepatocytes with clear vacuoles. These findings were consistent with clear cell hepatocellular carcinoma (CCHCC). The CCHCC is a rare subtype of hepatocellular carcinoma in dogs, and clinical features are poorly defined. This is the first report on the cytological, histological and clinical aspects of CCHCC, suggesting that obesity and hyperlipidemia are potential risk factors for CCHCC in dogs.

Keywords: clear cell; hepatocellular carcinoma; hyperlipidemias; obesity; case reports

Clear cell hepatocellular carcinoma (CCHCC) is a rare subtype of hepatic tumors that occur in less than 10% of dogs and is extremely rare in cats [1]. Histologically, the CCHCC comprises well-differentiated hepatocytes with vacuolated cytoplasm in hematoxylin and eosin (H&E) stained section. Generally, the vacuoles represent intracytoplasmic accumulation of glycogen and/or lipids [2,3]. In human medicine, CCHCC is considered as a low-grade malignancy with a favorable prognosis, which is unique when compared to other subtypes of hepatocellular carcinoma (HCC) [4,5]. However, in veterinary medicine, the incidence of CCHCC is rare, and thus clinical features of CCHCC have not been fully characterized. Here we describe the cytological, histological, and clinical aspects of CCHCC in a dog with obesity and hyperlipidemia.

An 11-year-old spayed female mixed-breed dog weighing 7.5 kg presented to a local hospital with elevated liver enzymes and increased total cholesterol levels (450 mg/dL; RI, 112 to 312 mg/dL). There was no evidence of other underlying diseases based on the exam. The patient received liver supplements for 1 month, but the liver enzyme levels remained high. After 2 months, the patient was referred to Seoul National University Veterinary Medical Teaching Hospital (SNU-VMTH) for further evaluation. At the time of admission to SNU-VMTH, the patient had no observable clinical signs. On physical examination, no remarkable findings were detected except that the body condition score (BCS) was 8/9.

The complete blood count (ADVIA2120i; Siemens Healthcare GmbH, Germany) showed mild thrombocytosis (70.4 × 10^4/μL; RI, 14.3 to 40.0 × 10^4/μL) and

neutrophilia (8.75 × 10^3/μL; RI, 3.9 to 8.0 × 10^3/μL). Serum biochemistry (Hitachi 7180; Hitachi High-Technologies Co., Japan) revealed a mild elevation of alanine aminotransferase (195 U/L; RI, 5.8 to 83.3 U/L), gamma-glutamyl transferase (31 U/L; RI, 0 to 14 U/L) and marked elevation of alkaline phosphatase (ALP, 2,375 U/L; RI, 0 to 97.9 U/L). Glucose (102 mg/dL; RI, 75 to 128 mg/dL), total bilirubin (0.2 mg/dL; RI, 0.1 to 0.5 mg/dL) and ammonia (24 μmol/L; RI, 16 to 75 μmol/L) were within the reference range. Computed tomography imaging revealed a mass, 1.84 × 1.65 × 2.6 cm in size with contrast enhancement, located on the right medial lobe of the liver. Based on the image analysis, malignant proliferative hepatic lesions were considered. No evidence of metastasis was found in other organs, including hepatic lymph nodes.

Fine-needle aspiration of the liver revealed clusters of hepatocytic epithelial cells (Fig. 1A). These cells were approximately 3 to 5 times of red blood cell (RBC) diameter and had moderate amounts of cytoplasm, which contains membrane-bound, discrete, and clear macro-vacuoles. The nuclei were round to oval, approximately 1.5 to 2 times of RBC diameter. The nuclei were displaced peripherally and flattened by the vacuoles (Fig. 1B). The nuclei had coarse chromatin and 1 to 2 small nucleoli in most cells. Anisocytosis and anisokaryosis were mild to moderate and mitotic figures were not observed (Fig. 1C). Occasionally, capillary vessels were coursing through the sheets of the hepatocytes (Fig. 1D). Lipids were strongly suspected for the vacuoles because of the distinct and refractile features. Based on the radiographic features and cytological findings, the diagnosis of well-differentiated HCC was made. Other differential diagnoses were benign hepatic lesions, including vacuolar hepatopathy or hepatic lipidosis.

Partial lobectomy of the right medial lobe of the liver was performed, and the tissue was subjected to histopathological evaluation (IDEXX Laboratories, USA). The liver specimens contained a coalescing irregular nodular mass devoid of portal architecture. Neoplastic hepatocytes compressed non-neoplastic hepatocytes near the capsule (Fig. 2A). The neoplastic cells had mildly pleomorphic nuclei and variably-sized vacuoles. The mitotic count was approximately 5 per 10 high power fields (Fig. 2B). We performed periodic acid-Schiff (PAS) staining to evaluate whether vacuolar components were glycogen or some other substance. Non-vacuolated neoplastic hepatocytes and non-neoplastic hepatocytes in the vicinity of the capsule contained abundant PAS-positive granules (Fig. 3A). However, most of the neoplastic hepatocytes with large vacuoles contained less PAS-positive granules (Fig. 3B). Based on the histopathology, a diagnosis of CCHCC was made because over 80% of hepatocytes were clear cells with vacuolated cytoplasm in H&E stain.

The patient was subjected to regular checkups to monitor liver enzyme levels and potential recurrence of the tumor, assessed via ultrasound. Six months after surgery, there was no evidence

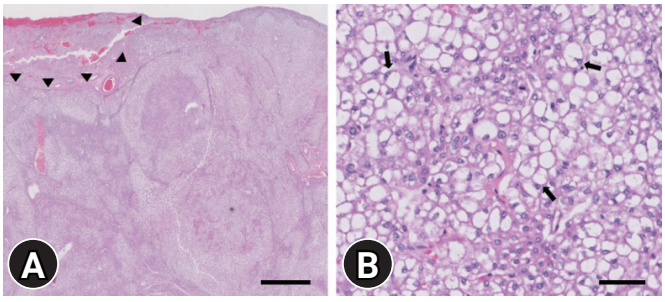

Fig. 2. Histopathology of the right medial liver lobe in a dog. (A) A nodular mass composed of vacuolated hepatocytes is devoid of portal architecture. Neoplastic hepatocytes (lower right side) compress non-neoplastic hepatocytes (upper left corner) near the capsule (arrowheads). (B) The neoplastic cells have mildly pleomorphic nuclei and variably-sized vacuoles which displace the nucleus peripherally (arrows). H&E, scale bar: (A) 1,200 μm, (B) 50 μm.

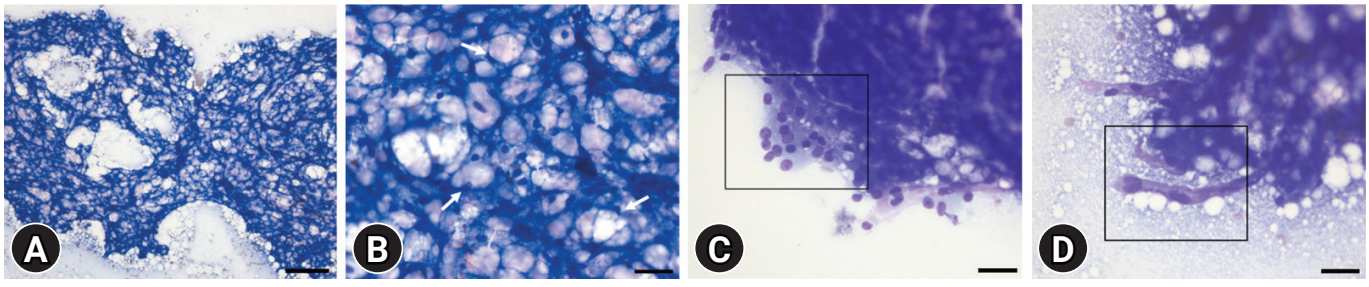

Fig. 1. Fine-needle aspiration from a mass on the right medial liver lobe in a dog. (A) Note the clusters of hepatocytes with variably-sized vacuoles. (B) The neoplastic hepatocytes have moderate amounts of cytoplasm with vacuoles that displace the nuclei (arrows). (C) Note the mild to moderate nuclear pleomorphism (black box) in the clusters of neoplastic cells. (D) Capillary vessels (black box) are coursing through the sheets of the hepatocytes. Diff-Quick, scale bar: (A) 100 μm, (B-D) 50 μm.

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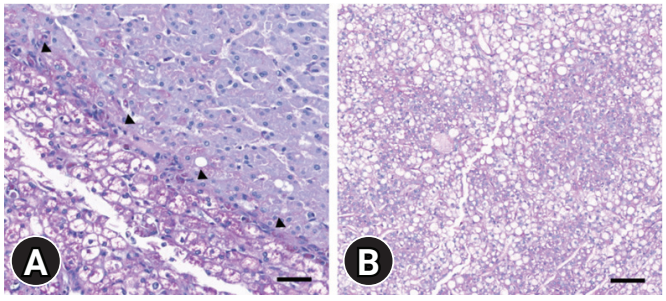

Fig. 3. Periodic acid-Schiff (PAS) staining of tissues from the right medial liver lobe in a dog. (A) Abundant PAS-positive materials are observed in non-vacuolated neoplastic hepatocytes (lower left) and non-neoplastic hepatocytes (upper right) in the vicinity of the capsule (arrowheads). (B) Note the neoplastic hepatocytes with large vacuoles containing a less amount of pink PAS-positive material. Scale bar: (A) 50 μm, (B) 100 μm.

of recurrence, but ALP was elevated further (from 1,727 to 3,009 U/L; RI, 0 to 97.9 U/L). Moreover, severe hyperlipidemia (triglyceride, 936 U/L; RI, 21 to 133 U/L) and hypercholesterolemia (385 mg/dL; RI, 112 to 312 mg/dL) were observed even after a 16-hour fasting period. The patient was prescribed antihyperlipidemic drugs, liver supplements and returned to the local hospital for treatment.

HCC is the most common liver tumor in dogs, accounting for approximately 50% of primary hepatic tumors [3]. It is classified into 4 major histologic subtypes, including trabecular, pseudoglandular, solid, and uncommon patterns, including scirrhous and clear cell types. The frequency of CCHCC in dogs is not well-established but relatively common in dogs than other species [2]. A study by Patnaik et al. [1] classified 5 out of 57 cases of canine HCC as a clear cell subtype. In humans, although no set of diagnostic criteria exist regarding the percentage of clear cells in CCHCC, if ≥ 50% of cell types are used as a cut-off, CCHCC accounts for 2.2% to 6.7% of all HCCs [6]. In the present case, most of the neoplastic cells had cytoplasmic vacuoles, and thus there was no difficulty in diagnosing CCHCC. Another important consideration for the diagnosis of CCHCC is the possibility of metastatic clear cell tumors, such as renal cell carcinoma [3,6]. In this case, tumors were not detected in any other organ on imaging analysis. Therefore, we could rule out the possibility of metastasis from other origins.

Compared to cats, glycogen accumulation in hepatocytes is common, but lipid vacuolation is relatively rare in dogs [7]. Cases of hepatic lipidosis were reported in dogs with aflatoxicosis [8] and metabolic disorders, including diabetes mellitus and idiopathic hyperlipidemia [9]. In this case, hyperlipidemia was present, but no other evidence of metabolic disease was found. Although it has been reported that dogs with hyperlipidemia also have hepatic lipidosis [10], no neoplastic lesions associated with hyperlipidemia have been reported. To the best of our knowledge, this is the first report of CCHCC in a dog with hyperlipidemia.

Considering obesity, indicated by BCS of 8/9 and severe hyperlipidemia in this patient, we could raise the question of whether obesity was a predisposing factor for developing CCHCC. In human medicine, studies suggested a positive rate of hepatitis C virus infection or liver cirrhosis as risk factors for CCHCC [6]. However, the relationship between obesity and CCHCC is not well understood. A study by Ko et al. [11] reported a CCHCC in a young woman with obesity, type II diabetes mellitus, and hyperlipidemia. Another case by Orikasa et al. [12] described a lipid-rich CCHCC in a slightly overweight patient with non-alcoholic steatohepatitis and diabetes mellitus. Moreover, Bannasch et al. [13] illustrated the pathogenesis and metabolic traits of CCHCC *in vitro* and suggested that lipidosis could provide metabolic precursors and enhance tumor development. Taken together, we postulate that obesity and idiopathic hyperlipidemia in our dog was associated with the development of CCHCC. Although fat metabolism in dogs is not identical to humans, this case could expand our knowledge about the risk factors of CCHCC in both human and veterinary medicine. Further investigations are warranted to determine the risk factors associated with CCHCC.

Although some reports describe the cytopathologic features of canine HCC [14,15], no cases are available for CCHCC in dogs. In this case, not only vacuolated cytoplasm but some capillaries were also seen, which are one of the cytologic features of well-differentiated HCC [14]. Because CCHCC is the well-differentiated neoplastic hepatocytes with vacuolated cytoplasm [5], cytological features in our case could help to differentiate CCHCC from benign lesions. To the author's knowledge, this is the first case to describe cytopathologic features of CCHCC that was diagnosed with a specimen from a liver lobectomy.

There are some limitations in this report. To confirm the lipid accumulation, special stains like Oil red O are required but could not be performed due to lack of available fresh tissue at the time of diagnosis. In the present case, the vacuoles were most likely lipid in nature because of negative staining for PAS and cytologic features of distinct, refractile vacuolation [7].

This is the first report to describe cytologic, histopathologic features and suggest the possibility of obesity and hyperlipidemia as risk factors of CCHCC in dogs. The CCHCC is a rare subtype of hepatic tumor in dogs and relatively unfamiliar in veterinary medicine. Because of the cytologic features of CCH-

CC, a presumptive diagnosis of benign lesions like vacuolar hepatopathy and hepatic lipidosis is most likely made using cytology alone. Limitations of hepatic cytology should be recognized, and for the definitive diagnosis, histological and imaging evaluation is essential. Additionally, we emphasize that more cases should be compiled to understand the characteristics of the CCHCC in veterinary medicine.

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