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Clinical and pathological features and outcome of bilateral incidental adrenocortical carcinomas in a dog

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ABSTRACT. A 9-year-old, spayed female Chihuahua was presented with a 1-week history of lethargy and anorexia. Abdominal ultrasonography and computed tomography found bilateral adrenal masses without metastasis. Serum cortisol levels that were sampled before and after an adrenocorticotropic hormone stimulation test were within reference ranges. Lethargy and anorexia completely resolved after short-term fluid therapy; the clinical signs did not occur for approximately 8 months until her sudden death. A postmortem examination revealed bilateral adrenocortical carcinomas and liver metastasis. Primary adrenocortical carcinomas developed in the dog met the definition of bilateral incidental adrenal gland masses (IAGMs). This is the first case report to demonstrate based on histological identification that adrenocortical carcinomas cause bilateral IAGMs in dogs.

KEY WORDS: adrenocortical carcinoma, dog, incidental adrenal gland mass

Incidental adrenal gland masses (IAGMs) are adrenal tumors that are accidentally discovered during abdominal imaging procedures while investigating an unrelated complaint [1, 9]. IAGMs in humans comprise of non-functioning, cortisol-secreting (subclinical Cushing's syndrome [SCS]) or aldosterone-secreting adenomas, pheochromocytoma, primary adrenocortical carcinomas, myelolipomas, cysts, metastases and various rare benign tumors [1, 9]. More than 70% of IAGMs in humans are benign non-functioning adenomas [1, 9]. Although IAGMs are usually discovered as unilateral IAGMs, bilateral IAGMs are also detected in up to 15% of patients with IAGMs [9, 10]. Bilateral IAGMs in humans develop likely due to metastatic disease, congenital adrenal hyperplasia, bilateral cortical adenomas or infiltrative disease [1, 9].

In veterinary medicine, advances in techniques in diagnostic sonography and ultrasound apparatus have enhanced the ability to detect IAGMs in dogs during routine abdominal ultrasonographic examinations. A recent study identified incidental adrenal gland lesions, including nodules, masses and nonspecific enlargements (≥10 mm), in 4% of dogs that underwent abdominal ultrasonography [5]. Another study also reported clinical characterization of dogs with non-cortisol-secreting adrenal masses, most of which were asymptomatic and corresponded to IAGMs [2]. Since pathological analysis was performed only in a few cases in these studies, the underlying cause for IAGMs remains to be determined in dogs.

As is the case in humans with IAGMs, bilateral IAGMs are uncommon in dogs. In a previous report, 11.3% of dogs with incidental adrenal gland lesions were bilateral in nature, including a dog with bilateral adrenal adenomas [5], although detailed characterization of the dog was not delineated. Here, we report clinical features, outcome and histopathological characteristics of a dog with bilateral incidental adrenocortical carcinomas.

A 9-year-old, spayed female Chihuahua weighing 2.3 kg was referred to Tokyo University of Agriculture and Technology Animal Medical Center for evaluation of a 1-week history of lethargy and anorexia. No clinical signs except lethargy and anorexia were detected at the initial presentation. A physical examination revealed no abnormality. Cardiac auscultation identified a grade III/VI systolic murmur over the mitral valve. A complete blood count exhibited an increase in the number of white blood cells (20.7×10^9 cells/*l*; reference range: $6.0–17.0 \times 10^9$ cells/*l*). A blood biochemical analysis detected an increase in the levels of aspartate aminotransferase (AST; 74 U/*l*, reference range: 17–44 U/*l*), alkaline phosphatase (ALP; 619 U/*l*, reference range: 47–254 U/*l*), C-reactive protein (CRP; 27.62 nmol/*l*, reference range: <6.67 nmol/*l*), and slight hypokalemia (3.5 mmol/*l*; reference range: 3.8–5.0 mmol/*l*). FUJI DRI-CHEM lipase (FDC-v-LIP) activity was mildly increased (270 U/*l*; reference range: 10–160

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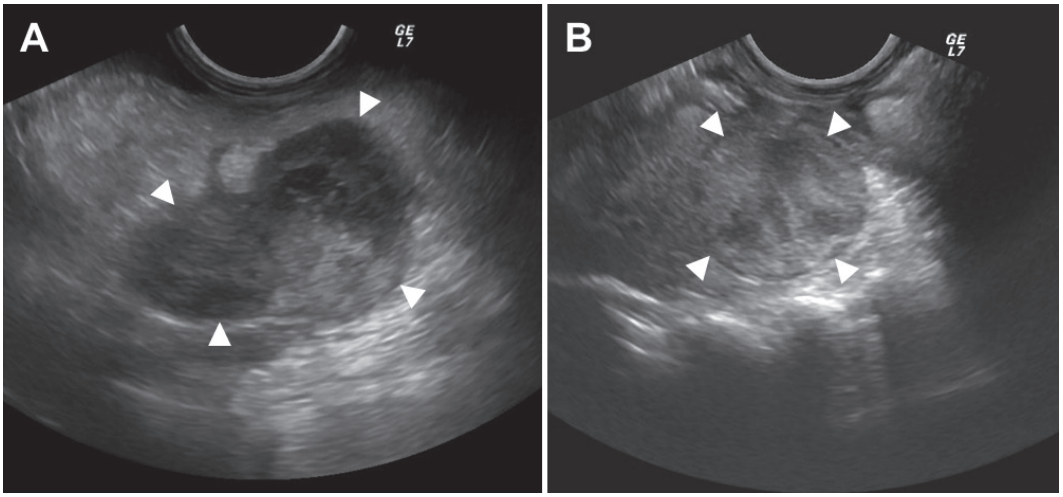

Fig. 1. Ultrasound images of both the adrenal glands. The left adrenal gland was an irregular shape with mixed echogenicity (A). The right adrenal gland was a round shape with mixed echogenicity (B).

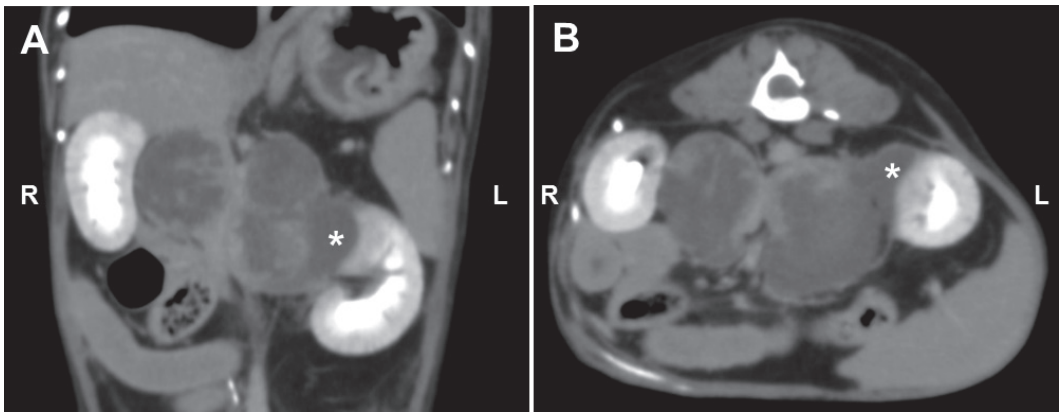

Fig. 2. Post-contrast computed tomography images of both the adrenal glands in the delayed phase. Dorsal plane (A) and transverse plane (B). Heterogeneous contrast effect was recognized in both the adrenal glands. There was hemorrhaging in the area of the renal pelvis (*). The sizes of the left and right adrenal glands were 25 × 30 × 42 mm and 25 × 31 × 26 mm, respectively. R, right; L, left.

U/*l*), to a value suggestive or indicative of pancreatitis [6]. Indirect blood pressure measurement demonstrated hypertension; systolic pressure was 187 mm Hg (reference range: <150 mmHg). Thoracic and abdominal radiography did not show any specific abnormality. Color Doppler echocardiography identified moderate mitral regurgitation. An abdominal ultrasound illustrated bilateral adrenal masses with mixed echogenicity (Fig. 1A and 1B). The left adrenal gland was an irregular shape (Fig. 1A), whereas the right adrenal gland was a round shape (Fig. 1B). Invasion of masses into the caudal vena cava was not detected. Since hyperadrenocorticism (HAC) was suspected, an adrenocorticotropic hormone (ACTH) stimulation test was performed. Serum cortisol concentrations were measured before (baseline) and 1 hr after administration of tetracosactide acetate (Cortrosyn; Daiichi-Sankyo, Co., Ltd., Tokyo, Japan, 0.125 mg, IM). Serum cortisol levels were 176.56 nmol/*l* at baseline (reference range: 27.59–215.19 nmol/*l*) and 317.26 nmol/*l* at 1 hr after ACTH stimulation (reference range: <551.76 nmol/*l*). To further examine both adrenal masses, the dog was inspected by computed tomography (CT) under general anesthesia. In the CT images (Fig. 2A and 2B), the sizes of the adrenal glands were 25 × 30 × 42 mm on the left side and 25 × 31 × 26 mm on the right side, respectively. The differentiation between the cranial and caudal parts of both the adrenal glands was difficult to discern because of its enlarged and irregular shape. The left adrenal gland was ruptured and hemorrhaged in the area of the renal pelvis (Fig. 2A and 2B). There was no invasion of tumors into the caudal vena cava. Since bilateral adrenal tumors were suspected based on the image findings, we proposed adrenalectomy for both the adrenal glands to the owner. However, adrenalectomy was not performed upon the owner's request.

The dog was hospitalized and treated with acetic acid Ringer's solution (Solacet® F; Terumo Corp., Tokyo, Japan) for lethargy and anorexia. The dog was also administered pimobendan (Pimobendan; Toa Eiyo Ltd., Fukushima, Japan, 0.25 mg/kg, PO, q12 hr) for mitral regurgitation and amlodipine (Amlodin; Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan, 0.25 mg/kg, PO,

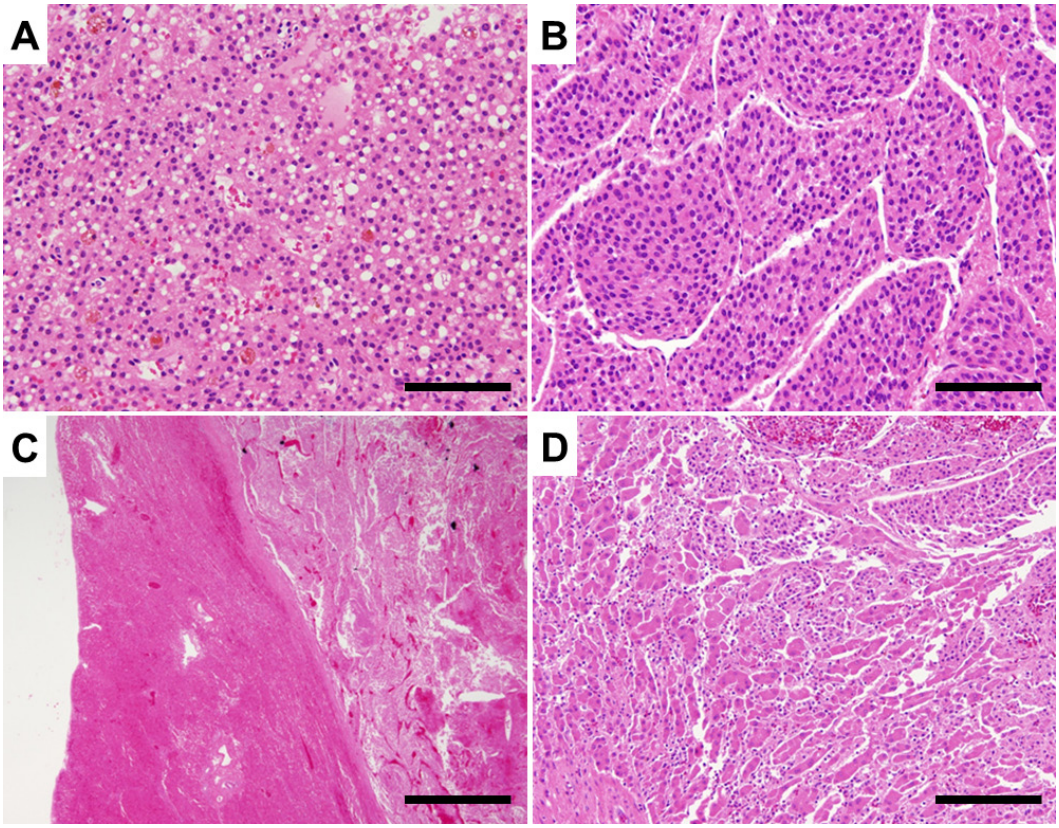

Fig. 3. Histopathological images of the tumors in both the adrenal glands and the liver. In the left adrenal gland, tumor cells with prominent cytoplasmic vacuolation proliferated in a solid growth pattern (A), while in the right adrenal gland, tumor cells with eosinophilic cytoplasm proliferated in a nest-like growth pattern (B). In the liver (C), tumor mass (right side) was encapsulated by fibrous connective tissues in the vicinity of liver parenchyma (left side), and tumor cells also invaded into the sinusoidal spaces and hepatic cords (D). Hematoxylin and eosin stain. Bar=50 (A, B), 100 (C) or 250 (D) μm.

q24 hr) for hypertension. On day 2, the dog was discharged due to complete improvement in activity and appetite. Treatment with pimobendan and amlodipine was continued after discharge. On day 28, no clinical signs, including lethargy and anorexia, were observed. A blood test was not performed. Blood pressures returned to the reference range. Therefore, amlodipine was discontinued, and only pimobendan was administered to the dog at the same dosage. On day 248, however, the dog suddenly died of an unknown cause. The dog had been active and had a good appetite, and had not showed any clinical signs until the day of death after discharge.

A necropsy found 10 m*l* of bloody ascites in the abdominal cavity. A dark red mass, approximately 40 mm in diameter, with hematoma was observed in the liver. The mass was observed in the basal portions of the right median lobe and caudate lobe in the liver. Both the adrenal glands were swollen and adhered to the hepatic mass. The histological examination revealed that multiple nodules of the tumor cells were scattered in both the adrenal glands. The nodules in the left adrenal gland were relatively larger than those in the right adrenal gland. In the left adrenal gland, the tumor cells were composed of round to oval nuclei and eosinophilic cytoplasm with variably-sized vacuoles, and were arranged in a solid growth pattern (Fig. 3A). No nuclear atypia and mitotic figures were detected; however, tumor cells compressed normal-appearing cortical and medullary tissues. There were on occasion foci of necrosis and hemorrhage in tumor tissues. In the right adrenal gland, the tumor cells were similar to those on the left side, but lacked cytoplasmic vacuolation, and were arranged in a nest-like growth pattern (Fig. 3B). In both the adrenal glands, the multiple nodules of tumor cells were demarcated by abundant connective tissues with infiltration of lymphocytes and plasma cells. Thus, the normal structures of cortical and medullary tissues were obscured. In the liver, the tumor tissues were demarcated by dense connective capsules (Fig. 3C) or scattered in the liver parenchyma (Fig. 3D). The tumor cells mimiced those in the right adrenal gland, with evidence of a nest-like growth pattern with necrosis and hemorrhage. The tumor cells in both the adrenal glands and the liver were further examined by immunohistochemical analysis, as described previously [8], with the primary antibody against chromogranin A (CGA; Yanaihara Institute Inc., Shizuoka, Japan), a neuroendocrine marker for pheochromocytoma [3]. The tumor cells in both the adrenal glands and the liver did not express chromogranin A, which tested positive in the adrenal medulla (data not shown). On the basis of histological and immunohistochemical analyses, both the adrenal masses were diagnosed with adrenocortical carcinomas, and the hepatic mass was a metastasis of adrenocortical carcinomas. It was also speculated that hemorrhage of the hepatic mass might have induced the sudden death.

The dog in this case report only showed a 1-week history of lethargy and anorexia. The dog did not exhibit typical clinical signs associated with HAC, including polyuria, polydipsia, polyphagia, skin lesions and pendulous abdomen. In addition, lethargy and anorexia completely resolved after short-term fluid therapy; the clinical signs did not occur for approximately 8 months until the death. These observations indicate that lethargy and anorexia were not related to the adrenal masses. The clinical signs might have been associated with possible pancreatitis as demonstrated by an increase in the levels of FDC-v-LIP and CRP. Although the underlying cause for elevated levels of AST and ALP were not determined, those might also have been induced by possible pancreatitis, but not the adrenal masses. Clinical history and the response to treatment indicate that the dog in this report met the definition of IAGMs.

The present case report provided histological evidence for the first time that adrenocortical carcinomas cause bilateral IAGMs in dogs. Adrenocortical carcinoma was identified to induce unilateral functional adrenal gland tumors in dogs [7]. Unilateral adrenocortical carcinoma was also histologically found in dogs with non-cortisol-secreting adrenal masses [2]. In humans with IAGMs, adrenocortical carcinoma is not frequently detected and account for 1.2 to 11% of IAGMs [1, 9]. The incidence of bilateral adrenocortical carcinomas in dogs and humans with IAGMs remains unclear. Although bilateral IAGMs are an uncommon disease in dogs, the current case report clearly demonstrates that adrenocortical carcinoma should be taken into consideration for the differential diagnosis of bilateral IAGMs in dogs.

Adrenocortical carcinomas are classified as being functional or non-functional based on hormone production from adrenal masses. It was not determined whether bilateral adrenocortical carcinomas in the dog in this report were functional or not. Serum cortisol levels before and after ACTH stimulation were within the reference intervals in the dog, suggesting that bilateral adrenocortical carcinomas might have been non-functional. However, because the dog showed elevated levels of ALP, slight hypokalemia and hypertension, bilateral IAGMs in the dog might have been functional, possibly due to cortisol-secreting SCS or primary aldosteronoma. SCS in humans is characterized by autonomous glucocorticoid production from adrenal masses without any specific clinical signs and symptoms [1]. To determine whether the dog had cortisol-secreting SCS, a low-dose dexamethasone suppression test (LDDST) may have been helpful, since the sensitivity and specificity of a LDDST are superior to that of an ACTH stimulation test [7]. Primary aldosteronoma, a very uncommon tumor in dogs, produces excess amount of mineralocorticoids and induces weakness, hypertension and hypokalemia. For diagnosis of aldosteronoma, plasma aldosterone concentrations and plasma renin activity should have been measured in the dog [4].

It might also be possible that bilateral IAGMs in the dog could have been atypical HAC or sex hormone-secreting adrenocortical carcinoma. To diagnose these diseases, 17-hydroxyprogesterone levels for atypical HAC, and progesterone, estradiol, testosterone and androstenedione levels for sex hormone-secreting adrenocortical carcinoma should be measured before and after administration of ACTH [4]. However, since the sensitivity and specificity of these tests are low [4], the diagnoses of these diseases may be difficult.

There is an algorithm for managing humans with IAGMs according to hormone production, size of tumor and imaging phenotype [1]. Recently, a diagnostic and therapeutic approach for IAGMs has been proposed in dogs [4]. If IAGMs are suspected to be malignant, i.e. masses are >2 cm in greatest diameter, vascular or tissue invasion or evidence of metastasis, adrenalectomy is recommended. In contrast, if IAGMs do not appear to be malignant, a non-surgical follow-up is suggested. The algorithms for IAGMs in humans and dogs are mainly based on unilateral IAGMs. There has been no guideline for managing bilateral IAGMs in humans and dogs. In the dog in this report, both the adrenal masses were >2 cm in greatest diameter. Histopathological analysis revealed bilateral adrenocortical carcinomas and hepatic metastasis. These findings indicate that irrespective of whether IAGMs develop bilaterally, if they are >2 cm in greatest diameter, they are strongly suggestive of malignant tumors in dogs. For malignant bilateral IAGMs in dogs, adrenalectomy for both the adrenal glands may be recommended.

Bilateral IAGMs could be metastatic from another primary tumor, as indicated in humans [1, 9]. In this case report, diagnostic imaging, including radiography, ultrasonography and CT, did not find any primary neoplasm except for the two adrenal masses. A postmortem examination detected bilateral adrenocortical carcinomas and hepatic metastasis. Thus, bilateral adrenocortical carcinomas in the dog are thought to have developed primarily in both the adrenal glands; those then invaded the liver over time. It is also possible that primary adrenocortical carcinoma developed in the right or left adrenal gland, metastasizing to the other adrenal gland and then to the liver. Histologically, the tumor cells in the right adrenal gland appeared to be less differentiated than those in the left adrenal gland. Accordingly, it can be speculated that primary cortical tumor cells in the right adrenal gland might have invaded the left adrenal gland and the liver tissues.

To the best of the authors' knowledge, this is the first case report to demonstrate based on histological identification that adrenocortical carcinomas cause bilateral IAGMs in dogs. IAGMs have been increasingly diagnosed in dogs. Although most IAGMs are detected as unilateral IAGMs, it is important to note that bilateral IAGMs can develop in dogs as an uncommon type of IAGMs. Considering clinical and pathological features and outcome of the dog in this report, a recently proposed diagnostic and therapeutic approach for IAGMs in dogs may be applied to bilateral IAGMs.

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