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< Short Communication >

Primary pheochromocytoma in an Asian Water Buffalo (*Bubalus bubalis*)

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Abstract

A mass of the adrenal gland was observed during a routine necropsy of a female 23-year-old Asian Water Buffalo (*Bubalus bubalis*) at Seoul Zoo in Gyeonggi Province, Korea. The animal showed no clinical signs but the necropsy examination revealed hydropericardium, ascites, hydrothorax and edema of the intestinal wall, lung and adrenal gland. Histopathologically, the neoplastic cells of the right adrenal gland were arranged in lobules supported by a fine fibrovascular stroma. The neoplastic cells had round hyperchromatic nuclei and granular eosinophilic to basophilic cytoplasm. Immunohistochemically, tumor cells were positive for chromogranin A and S-100 and negative for vimentin, synaptophysin and cytokeratin. Based on the above findings, this case was diagnosed as a pheochromocytoma. To the best of our knowledge, this is the first report of a pheochromocytoma in an Asian Water Buffalo (*Bubalus bubalis*).

Key words : Adrenal gland, Pheochromocytoma, Asian Water Buffalo, Zoo animal

INTRODUCTION

Pheochromocytoma is the most common tumor of the medulla of the adrenal glands originating in the chromaffincells, which secretes excessive amounts of catecholamines, usually epinephrine and norepinephrine (Meuten, 2002). They may be unilateral or bilateral and single or multiple. Pheochromocytomas are generally enlarged and replaced affected adrenal gland and small ones are completely surrounded by compressed adrenal cortex. They have been reported most frequently in cattle and dogs and rare in other domestic animals (Meuten, 2002), some strains of laboratory rats (Sheehy et al, 1997) and wild animals, which include a wolfdog (Sako et al, 2001), a Nicobar Pigeon (*Caloenas nicobarica*) (Sonnenfield et al, 2002), callitrichids (Garner, 2001) and spotted dolphins (*Stenella frontalis*) (Martineau et al, 2002). Adrenal tumors are very rare in wild ruminants. There is one adrenal cortical carcinoma report available in a free ranging mouflon previously (Marco-Sánchez et al, 1996).

Functional pheochromocytoma has been reported infrequently in animals (Bouayad et al, 1987; Cuervo et al, 1994). In dog, this tumor, appear to be rare, constituting only 0.13～0.01% of all canine tumors (Meuten, 2002), but they are the second most common adrenal neoplasm in dogs (Massari et al, 2011). Pheochromocytomas are benign histologically, slow-growing, potentially functional, and noninvasive neoplasms (Meuten, 2002). They are occasionally malignant with metastasis to distant organs (Massari et al, 2011). Clinical signs are usually related to the catecholamine release, and it's depending of the kind of receptors and adrenergic classes stimulated (Prys-Roberts, 2000). Pheochromocytomas occasionally result in secondary conditions like thrombosis of the caudal vena cava and aortic thromboembolism, which occurs due to the endothelial dam-

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age, blood stasis and hypercoagulable states (Gonçalves et al, 2008).

Here, we report a rare case of pheochromocytoma in the Asian Water Buffalo along with the histopathological and immunohistochemical features. To the best of our knowledge, there are no reports of pheochromocytoma and its immunohistochemical features in the the Asian Water Buffalo.

CASE REPORT

A 23-year-old female Asian water buffalo was found dead at Seoul Zoo in in Gyeonggi Province, Korea. The gross findings were hydrothorax, ascites, hydropericardium and edema of the intestinal wall and lung, kidney and adrenal gland. When the adrenal medulla was cut a very large portion of medullary tissue was observed against a apparently thin cortical tissue (Fig. 1). This observation was noted in both adrenal glands (bilateral). The other lesion noted was in both kidneys with multiple cysts. The liver was very congested and bulged on the cut surface. The spleen was firm and hard with plenty of fibrous connective tissue resulting in a whitish external coloration. There were no other visible lesions in the other organs. The tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin.

Serial sections of adrenal glands were examined immunohistochemically using the avidinbiotinperoxidase complex (ABC) procedure (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA). Mouse monoclonal antibodies against chromogranin A (CGA) (1:500, Dakocytomation, Glostrup, Denmark), S-100 (prediluted product, Dakocytomation, Glostrup, Denmark), vimentin (prediluted product, Dakocytomation, Glostrup, Denmark), synaptophysin (SYN) (1:100, Dakocytomation, Glostrup, Denmark), and cytokeratin AE1/AE3 (CK, prediluted product, Dakocytomation, Glostrup, Denmark) were used as primary antibodies. The endogenous peroxidase activity in the deparaffinized sections was blocked using 3% H_2O_2 for 10 minutes. All sections were incubated with the primary antibody at 4°C for 16 hours, with biotinylated secondary antibody for 30 minutes at room temperature, and with avidinperoxidase conjugate for 30 minutes. Staining was developed in a 0.05% 3, 3'-diaminobenzidine solution. Appropriate positive and negative controls were performed. In the negative control, primary antibody was replaced by normal serum.

Histopathologically, the neoplastic cells of the right adrenal gland were arranged in lobules supported by a fine fibrovascular stroma (Fig. 2). The neoplastic cells had round hyperchromatic nuclei and granular eosinophilic to basophilic cytoplasm (Fig. 3). Mitotic figures

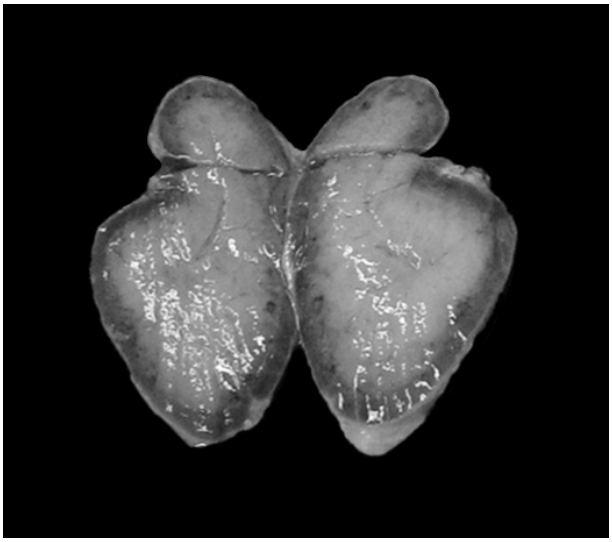

Fig. 1. Gross lesion of adrenal gland, Asian buffalo. Multifocal masses were seen in the thin adrenal cortex.

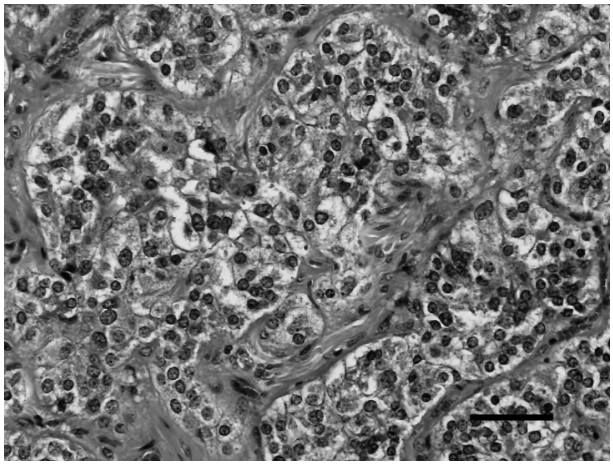

Fig. 2. Adrenal gland pheochromocytoma, Asian buffalo. The neoplastic cells are arranged in small lobules supported by a fine fibrovascular stroma and have round nuclei and granular cytoplasm. H&E stain. Bar=50 μm.

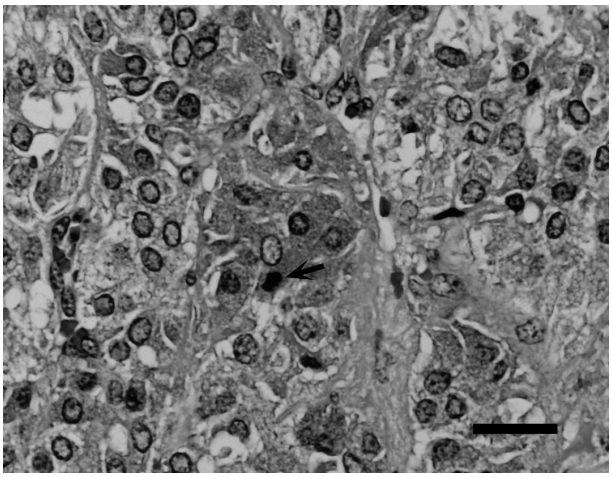

Fig. 3. Adrenal gland pheochromocytoma; Asian buffalo. Most neoplastic cells had round hyperchromatic nuclei and granular eosinophilic to basophilic cytoplasm. Mitotic figures were found frequently (arrow). H&E stain. Bar=25 μm.

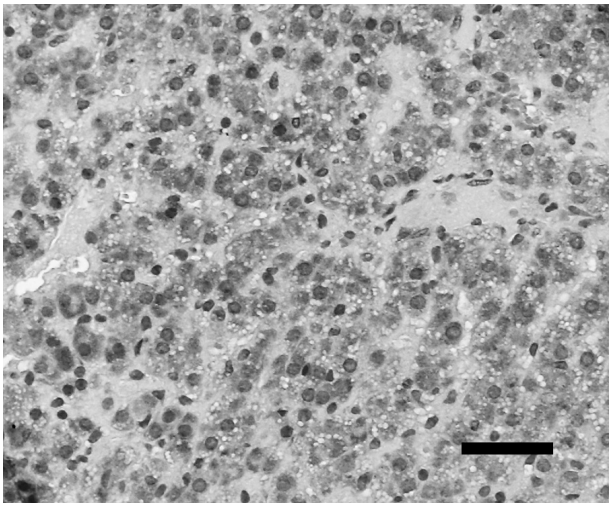

Fig. 4. Adrenal gland pheochromocytoma, Asian buffalo. Most neoplastic cells react positively for the presence of chromogranin A. ABC method, Mayer's hematoxylin counterstain. Bar=50 μm.

were found frequently (Fig. 3). Immunohistochemically, tumor cells were positive for CGA (Fig. 4) and S-100 (Fig. 5) and negative for vimentin, SYN and CK (data not shown).

This tumor was diagnosed both histologically and immunohistochemically as a malignant pheochromocytoma in an Asian Water Buffalo.

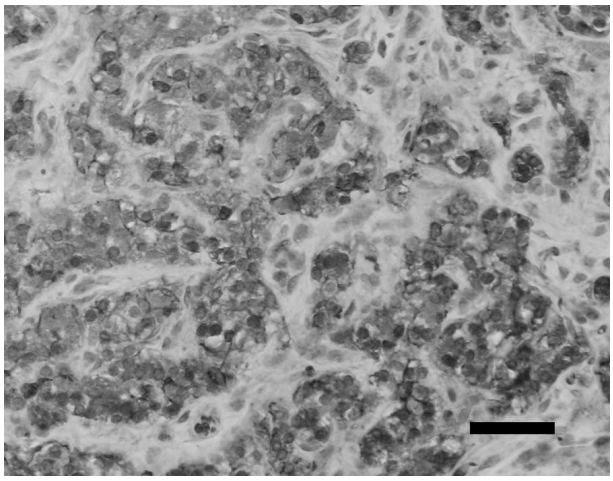

Fig. 5. Adrenal gland pheochromocytoma, Asian buffalo. Most neoplastic cells react positively for the presence of S-100. ABC method, Mayer's hematoxylin counterstain. Bar=50 μm.

DISCUSSION

A differential diagnosis of adrenal gland neoplasms comprises adrenal gland hyperplasia. adenoma, pheochromocytoma, neuroblastoma and ganglioneuroma. Comparative histopathology with tumors of the adrenal gland strongly indicated that the histopathological features were compatible with a pheochromocytoma. The necropsy findings such as the systemic edema (ascitis and hydropericardium), liver congestion and intestinal edema might have been the result of a possible functional pheochromocytoma. Although no attempt was made to quantify the amount of epinephrine or norepinephrine when the animal was alive due to absence of clinical signs, the necropsy finding of systemic edema as a result of overstimulation of the circulatory system, which most likely caused tachycardia, might have contributed to the general congestive condition of the animal that resulted in the systemic edema observed during the necropsy.

Immunohistochemistry is commonly used to differentiate adrenal medullary tumors from cortical or extra-adrenal tumors (McNicol, 2006). Markers commonly used to differentiate pheochromocytomas include CGA, SYN and neuron-specific enolase (NSE) (Feldman and Eiden, 2003; McNicol, 2006). Neuroendocrine markers like CGA, NSE and SYN are typically positive in cases of pheochromocytoma, particularly with CGA, which is

reported to stain 100% of pheochromocytomas (McNicol, 2006). CGA is the major secreted protein of the adrenal medulla and play an important role in binding and aggregating intracellular calcium (Feldman and Eiden, 2003). In addition, there are defined roles for chromogranins in functioning as prohormones, molecular chaperones, and modulators of gene expression (Feldman and Eiden, 2003). SYN is a membrane glycoprotein found in prejunctional neuroendocrine granules and is used as a specific marker of neuroendocrine tumors (McNicol, 2006).

In a wolf dog, a large number of the neoplastic cells in the primary neoplasm and the metastases were strongly positive for CGA, SYN, and substance P (SP). A small number reacted to S-100 protein (Sako et al, 2001). However, the neoplastic cells were negative for glial fibrillary acid protein, neurofilament, vimentin, α-smooth muscle actin, and cytokeratin (Sako et al, 2001). In this case, tumor cells showed positive for CGA (Fig. 4) and S-100 (Fig. 5) and negative for SYN. These results are similar to previos reports.

Malignant pheochromocytomas have a thin fibrous capsule that is invaded at several points. They exert pressure on the posterior vena cava or infiltrate the vessel, forming a tumor cell thrombus (Meuten, 2002). In the present case, histological aspects of the cells were uniform and moderate mitotic activity was observed. Although no metastases were detected in other organs, particularly in lungs, it was considered as malignant.

Functional pheochromocytomas produce norepinephrine, both norepinephrine and epinephrine, or, less commonly, dopamine. Overproduction of catecholamines by a pheochromocytoma may be episodic or continuous depending on the secretory pattern of the tumor. Catecholamine excess may cause a myriad of physiologic changes, the most common of which is systemic hypertension (Meuten, 2002). Functional pheochromocytomas have been reported infrequently in animals. Tachycardia, edema, and cardiac hypertrophy observed in several dogs and horses with pheochromocytomas were attributed to excessive catecholamine secretion (von Dehn et al, 1995).

In this case, there was no clinical signs prior to death. Nevertheless, on the necropsy, pulmonary edema, edema of serous surface of abdominal cavity, hydrothorax and ascites were seen. These lesions were similar to human pheochromocytoma case (Kondo et al, 2004).

The failure to show obvious clinical signs in wild animals even though the animal is sick is a defensive self-preservation behavior. Animals showing signs of weakness are prone to predation. With the above findings, this case was concluded to be a classical functional pheochromocytoma of the adrenal gland.

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