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Short Communication

Multiple diagnostic approaches in the rare case of pheochromocytoma in a non-descriptive dog

N. Pazhanivel[1*], M. Shafiuzama[2], M. Ali[2], K. Jeyaraja[3], B. Behera[1], H. R. Sushma[1] and G. V. Sudhakar Rao[1]

[1]Department of Veterinary Pathology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600 007, Tamil Nadu, India; [2]Department of Veterinary Surgery and Radiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600 007, Tamil Nadu, India; [3]Department of Veterinary Clinical Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai- 600 007, Tamil Nadu, India

Abstract

Pheochromocytoma is a tumor of the chromaffin cells of adrenal medulla. A 7 years old female non-descriptive dog was presented with a history of dullness and depression. Abdominal swelling was observed in physical examination. Ultrasonography revealed right adrenal gland enlargement with heterogenous parenchyma and highly vascular. Grossly, uniform enlargement of the right adrenal gland with a round to spherical mass and microscopical examination showed embedded mass consisting of single to large nests of neoplastic cells with fibrous stroma and atypical blood vessels. Large polygonal cells with basophilic, highly granular cytoplasm contain hyperchromatic vesicular nuclei with prominent nucleoli, and mitotic figures were also observed. Immunohistochemical examination by using chromogranin-A revealed moderate to strong cytoplasmic staining in the neoplastic cells, whereas synaptophysin showed mild brown cytoplasmic reaction. By using S100 marker showed moderate expression in sustentacular cells. Proliferation marker of Ki-67 showed strong expression. Based on gross, ultrasonography, histopathology and immunohistochemical examination, the mass was confirmed as pheochromocytoma. This is a rare case recorded in non-descriptive dogs by performing different diagnostic methods of abdominal ultrasonography and immunohistochemistry using specific marker of chromogranin-A along with gross and histopathology. Chromogranin-A is a specific marker for the pheochromocytoma which was showed positive in the tumor mass. The treatment of adrenalectomy was performed to prevent further occurrence.

Key words: Immunohistochemistry, Non-descriptive dog, Pathology, Pheochromocytoma, Ultrasonography

Highlights

- Rare case of pheochromocytoma was recorded in Non-descriptive dog.
- Immunohistochemical and ultrasonographical evaluation of the pheochromocytoma was performed.

Pheochromocytomas are tumors originating from the chromaffin cells of the adrenal medulla (Musser *et al.*, 2018) and they are catecholamine-producing neuroendocrine tumors (Salesov *et al.*, 2015). It is one of the frequent neoplasms of the adrenal gland in dogs (Kiupel *et al.*, 2008). These tumors are mostly unilateral and identified as large masses completely occupying the gland and less frequently as small nodules. It is more aggressive in dogs compared to humans (Barthez *et al.*, 1997). Physical examination of the animals affected with pheochromocytoma revealed systemic arterial hypertension and cardiac arrhythmias (Barthez *et al.*, 1997; Kiupel *et al.*, 2008). It often invades the adrenal capsule and invasion the adjacent tissues of the caudal vena cava developing large thrombi which would partially or completely occlude the vein leading to an abdominal effusion (Thompson, 2002). Sudden weakness and death might be due to bleeding from the tumor mass or injured vessels

*Corresponding Author, E mail: drnpvel@gmail.com

in pheochromocytoma affected dogs (Whittemore *et al.*, 2001). Pheochromocytomas are usually considered to be malignant due to invasion through the capsule in the adjacent tissues or metastasis to a distant site. In 39-50% of cases, these tumors are invasive, and it can be distant metastasis in 13 to 24% of cases with metastasis in different organs like a lymph node, liver, lung, kidney spleen and bone (Barthez *et al.*, 1997). The prognosis of pheochromocytoma affected dogs along with the survival time of dogs following adrenalectomy is mostly associated with surgical factors based on the local invasion or distant metastasis having prognostic relevance (Barthez *et al.*, 1997) and the mean survival time is more than 3 years after discharge from the hospital. In addition to that, most cases lost their follow-up or death due to other diseases (Barthez *et al.*, 1997) and favourable outcomes were observed at a young age with a shorter duration of surgery (Pappachan *et al.*, 2014). One of the poor outcomes associated with this tumour was caudal vena cava invasion leading to death (Gostelow *et al.*, 2013). The World Health Organization classification scheme may not predict the malignant behaviour of this tumor in dogs by using morphological criteria of tumor cells (Kiupel *et al.*, 2008). In addition to that in humans, pheochromocytoma behaviour was debated due to no morphological criteria has been identified the malignant behaviour of tumors (Thompson, 2002).

In this regard, human cases of pheochromocytoma have been diagnosed by the use of histological scores of different microscopic features (a combination of 12-pheochromocytoma of the adrenal gland scaled score) and use immunohistochemistry (Thompson, 2002; Yamamoto *et al.*, 2013). Based on the above information, the present case study was undertaken to assess the rare case of pheochromocytoma based on multiple diagnostic approaches using abdominal ultrasonography, gross pathology, histopathology, special staining and immunohistochemical markers expression in a seven-year-old non-descriptive dog.

A seven-year-old female non-descriptive dog was presented to the Madras Veterinary College Teaching Hospital with a history of dullness, depression, reduction in body weight and anorexia in past days. Physical examination of the animal revealed the swelling of the abdomen. Biochemical examination revealed elevation of glucose (579 mg/dL), slight elevation of cholesterol (331mg/dL), and other parameters like total protein, albumin, BUN, creatinine, ALT, GGT, ALP, total bilirubin, direct bilirubin, calcium, and phosphorus were within the normal range. Haematological values showed leucocytosis (22500/cm^3) and differential leukocyte count showed neutrophilia (83%). Abdominal ultrasonography examination revealed severe enlargement of the right adrenal gland with heterogeneous parenchyma (3.8×2.9 cm), highly vascular and impinging on the posterior vena cava, and this impression suggestive of right adrenal tumor (Fig. 1A).

The excised mass was collected in 10% neutral buffered formalin for histopathological examination. Paraffin embedded tissue sections were cut into 4-5 micron thickness and stained with Haematoxylin and Eosin (H&E) and Masson's Trichrome staining as per the procedure described by Bancroft and Gamble (2013), and subsequent microscopy was carried out with Olympus BX-51 Microscope with image analyser-Mag Vision software. The paraffinized tissue sections were subjected to IHC using markers chromogranin-A, synaptophysin, S-100 and Ki-67 as per the procedures described by the manufacturer's kit for the conformation of the tumor.

The gross examination of the right adrenal gland mass revealed uniform enlargement with a round to spherical shaped measuring about 4x3 cm (Fig. 1B), and the cut section revealed soft to firm light grey white coloured mass with multifocal haemorrhages and ulcers (Fig. 1C).

Microscopic examination of the mass revealed that the mass was embedded without breaking the capsule of the adrenal gland. The neoplastic cells were single to large nests with moderate to abundant fibrous stroma and atypical to well formed blood vessels, focal

haemorrhage and necrosis were also observed (Fig. 2A & 2B). The neoplastic cells were large polygonal shaped cells with eosinophilic to basophilic finely granular cytoplasm. Nuclei were enlarged hyperchromatic round to ovoid vesicular with a prominent single to multiple nucleoli (Fig. 2C). Atypical mitotic figures of more than one in 10 high power fields were observed. Masson's trichrome staining revealed the presence of fibrous tissue as blue colour surrounds the neoplastic nests (Fig. 2D).

The expression of chromogranin was observed as a moderate to strong brown coloured positive cytoplasmic reaction in the neoplastic cells (Fig. 3A). Similarly, synaptophysin was also expressed as the mild brown coloured cytoplasmic reaction in the neoplastic cells (Fig. 3B). The moderate expression of the S100 marker was also observed in sustentacular cells of the mass (Fig. 3C). The proliferation marker of Ki-67 showed a strong nuclear positive reaction in the neoplastic cells (Fig. 3D).

Based on the gross, ultrasonographical, microscopical and immunohistochemical examination of the mass confirmed the case of pheochromocytoma. Pheochromocytoma incidence found in all canine tumors with a percentage of 0.01 to 0.1 (Ware and Hopper, 1999). Due to this less incidence based on the autopsy incidental findings while the biochemical diagnosis has become possible in recent years (Gregori *et al.*, 2015). The age of the affected dogs with pheochromocytoma ranges from 1.6 to 18 years, whereas in the present case, the dog was 7 years old (Barthez *et al.*, 1997). The present case was approximately measured as a 4x3 size large mass, while the average diameter of this tumor was 5.1 cm (1.1-15 cm) (Gregori *et al.*, 2015). Most of the pheochromocytoma recorded in dogs are unilateral with a local invasion of frequency of 56% (Barrera *et al.*, 2013), whereas in the present case also recorded on the unilaterally right side. Clinical signs of weakness, abdominal discomfort, panting, arrhythmia and collapse (Barthez *et al.*, 1997). However, the clinical signs of the present case are also in accordance with the previous reports, while the abdominal distension might be due to the space occupying masses (Barthez *et al.*, 1997). In the present case, no metastasis was observed, whereas dogs with metastatic disease revealed associated clinical signs pertaining to the site of metastasis (Berzon, 1981). Haematobiochemical findings of the present case showed leucocytosis, neutrophilia, hyperglycaemia, and hypercholesterolemia which are observed in earlier reports (Barthez *et al.*, 1997). But the change in haematobiochemical values is not having great importance regarding diagnosis. Invasiveness of PCC is mainly due to occlusion of caudal vena cava (Santamarina *et al.*, 2003), however in the present case, the same findings were observed in ultrasonography. Abdominal ultrasonography is one of the routinely used diagnostic imaging techniques for pheochromocytoma and mostly is recorded incidentally. Histologically, PCC is characterized by polygonal basophilic neoplastic cells with rounded hyperchromatic nuclei arranged in a nest surrounded by fibrovascular stroma (Galac and Korpershoek, 2017). In the present case also, the histological features are similar to previous reports. TNM classification has been applied in 2 large scale studies in canine pheochromocytoma (Galac and Korpershoek, 2017). Diagnosis of PCC is supported by immunohistochemistry using chromogranin A, synaptophysin and neuron specific enolase and they are stains the chromaffin cells with S100 specific for sustentacular cells (Pacak, 2007). In the present case also the markers of chromogranin-A and synaptophysin showed positive expression indicating that most specific markers for diagnosis of PCC (Galac and Korpershoek, 2017). S100 expression was observed in the sustentacular cells of the present case. The preferred treatment for PCC is adrenalectomy due to the removal of PCC causing the reversal of all the clinical signs and symptoms and uncontrolled growth complications (Pacak,

Pheochromocytoma in non-descript dog

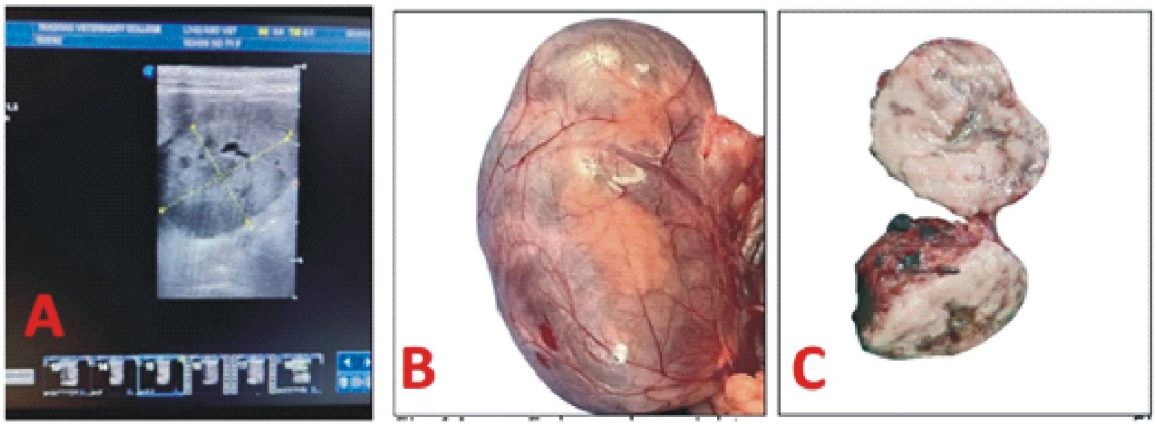

Fig. 1. A. Ultrasonography- Severe enlargement of right adrenal gland with heterogenous parenchyma (3.8 x 2.9 cm), highly vascular and impinging on the posterior vena cava; B. Enlarged right adrenal gland revealed round to spherical shaped mass measuring about 4 x3 cm; C. Cut section revealed soft to firm light grey white coloured mass

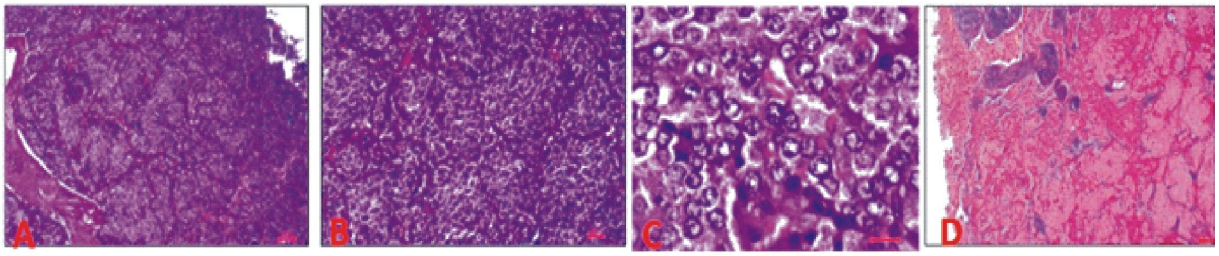

Fig. 2. A&B. Neoplastic cells were arranged in single to large nests with moderate to abundant fibrous stroma and atypical to well formed blood vessels, H&E Scale bar 20 µm; C. Neoplastic cell nuclei having prominent multiple nucleoli, H&E 1000x; D. Presence of fibrous tissue surrounding the tumor nests Masson's Trichrome stain Scale Bar 50 µm

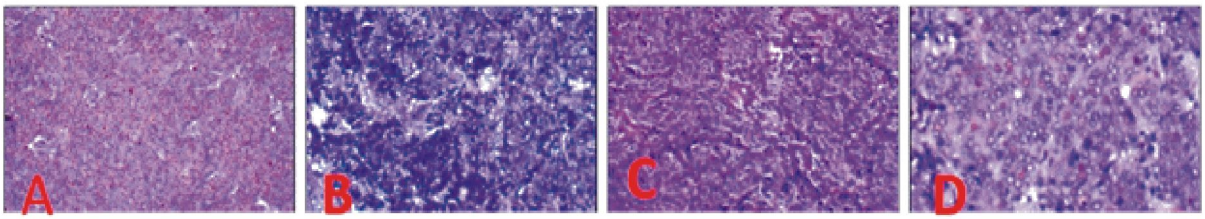

Fig. 3. A. Moderate to strong brown cytoplasmic reaction in the neoplastic cells Chromogranin Scale Bar 50 µm; B. Mild brown cytoplasmic reaction in the neoplastic cells Synaptophysin Scale Bar 20 µm; C. Moderate expression in sustentacular cells of the mass S 100 Scale Bar 20 µm; D. Mild to moderate nucleus expression in the neoplastic cells Ki67 Scale Bar 10 µm

2007), while the adrenalectomy was performed in the present case.

In the present study, PCC was diagnosed by histopathological and immunohistochemical methods without distant metastasis. The neoplastic cells may be contained neuropeptides such as chromogranin-A, substance P, SYN, Leu-7, PGP 9.5 (Protein gene product), ME (Methionine-Enkephalin), S100 protein, and GAL (Galanin) (Sako *et al.*, 2001). The result indicated that the present neoplastic cells also had neuropeptides of chromogranin A, synaptophysin and S100 which are specific immunochemistry markers for PCC.

PCCs are rare tumors in dogs and diagnosis of this tumor is also challenging. Diagnosis can be done by use of ultrasonography, histopathological and immunohistochemical method. The best method for treatment is surgical removal of tumor mass.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: NP: Methodology; MS: Conceptualization; UMA: Investigation; KJ: Formal analysis; BB: Editing; HRS: Revision of the manuscript; GVSR: Data curation.

REFERENCES

Bancroft JD and Gamble M, 2013. Microorganisms. Theory and Practice of Histological Techniques, 7th edn., Churchill Livingstone, London

Barrera JS, Bernard F, Ehrhart EJ, Withrow SJ and Monnet E, 2013. Evaluation of risk factors for outcome associated with adrenal gland tumors with or without invasion of the caudal vena cava and treated via adrenalectomy in dogs: 86 cases (1993–2009). J Am Vet Med Assoc, 242(12): 1715-1721, doi: 10.2460/javma.242.12.1715

Barthez PY, Marks SL, Woo J, Feldman EC and Matteucci M, 1997. Pheochromocytoma in dogs: 61 cases (1984-1995). J Vet Intern Med, 11(5): 272-278, doi: 10.1111/j.1939-1676.1997.tb00464.x

Berzon JL, 1981. A metastatic pheochromocytoma causing progressive paraparesis in a dog. Vet Med Small Anim Clin, 76(5): 675-679

Galac S and Korpershoek E, 2017. Pheochromocytomas and paragangliomas in humans and dogs. Vet Comp Oncol, 15: 1158-1170, doi: 10.1111/vco.12291

Gostelow R, Bridger N and Syme HM, 2013. Plasma-free metanephrine and free normetanephrine measurement for the diagnosis of pheochromocytoma in dogs. J Vet Intern Med, 27(1): 83-90, doi: 10.1111/jvim.12009

Gregori T, Mantis P, Benigni L, Priestnall SL and Lamb CR, 2015. Comparison of computed tomographic and pathologic findings in 17 dogs with primary adrenal neoplasia. Vet Radiol Ultrasound, 56(2): 153-159, doi: 10.1111/vru.12209

Kiupel M, Capen C and Miller MR, 2008. Histological Classification of Tumors of the Endocrine System of Domestic Animals. Vol 12. Washington, DC: Armed Forces Institute of Pathology– World Health Organization

Musser ML, Taikowski KL, Johannes CM and Bergman PJ, 2018. Retrospective evaluation of toceranib phosphate (Palladia®) use in the treatment of inoperable, metastatic, or recurrent canine pheochromocytomas: 5 dogs (2014-2017). BMC Vet Res, 14(1): 272, doi: 10.1186/s12917-018-1597-7

Pacak K, 2007. Preoperative management of the pheochromocytoma patient. J Clin Endocrinol Metab, 92(11): 4069-4079, doi: 10.1210/jc.2007-1720

Pappachan JM, Raskauskiene D, Sriraman R, Edavalath M and Hanna FW, 2014. Diagnosis and management of pheochromocytoma: A practical guide to clinicians. Curr Hypertens Rep, 16(7): 442, doi: 10.1007/s11906-014-0442-z

Sako T, Kitamura N, Kagawa Y, Hirayama K, Morita M *et al.*, 2001. Immunohistochemical evaluation of a malignant pheochromocytoma in a wolfdog. Vet Pathol, 38(4): 447-450, doi: 10.1354/vp.38-4-447

Salesov EG, Boretti FS, Sieber-Ruckstuhl NS, Rentsch KM, Riond B *et al.*, 2015. Urinary and plasma catecholamine and metanephrines in dogs with pheochromocytoma, hypercortisolism, nonadrenal disease and in healthy dogs. J Vet Intern Med, 29(2): 597-602, doi: 10.1111/jvim.12569

Santamarina G, Espino L, Vila M, Lopez M, Aleman N *et al.*, 2003. Aortic thromboembolism and retroperitoneal haemorrhage associated with a

pheochromocytoma in a dog. J Vet Intern Med, 17(6): 917-922, doi: 10.1111/j.1939-1676.2003.tb02533.x

Thompson LDR, 2002. Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: A clinicopathologic and immunophenotypic study of 100 cases. Am J Surg Pathol, 26: 551-566, doi: 10.1097/00000478-200205000-00002

Ware WA and Hopper DL, 1999. Cardiac tumors in dogs: 1982–1995. J Vet Intern Med, 13(2): 95-103, doi: 10.1892/0891-6640(1999)013<0095: ctid>2.3.co;2

Whittemore JC, Preston CA, Kyles AE, Hardie EM and Feldman EC, 2001. Nontraumatic rupture of an adrenal gland tumor causing intra-abdominal or retroperitoneal haemorrhage in four dogs. J Am Vet Med Assoc, 219(3): 329-333, doi: 10.2460/javma.2001.219.329

Yamamoto S, Fukushima R, Hirakawa A, Abe M, Kobayashi M *et al*., 2013. Histopathological and immunohistochemical evaluation of malignant potential in canine aortic body tumours. J Comp Pathol, 149(2-3): 182-191, doi: 10.1016/j.jcpa.2012.12.007

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