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S Subapriya
Assistant Professor, Centralized Clinical Laboratory, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

N Pazhanivel
Professor, Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

S Ramesh
Professor, Department of Veterinary Pathology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Mohammed Ali
Assistant Professor, Department of Clinics, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

D Sumathi
Assistant Professor, Department of Veterinary Clinical Medicine and Therapeutics, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

M Sandhya Bhavani
Assistant Professor, Department of Clinics, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Reeta Bascal
Chief Technician, Central Electron Microscopy Facility, Wellcome Laboratory, Christian Medical College, Vellore, Tamil Nadu, India

S Vairamuthu
Professor and Head, Centralized Clinical Laboratory, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Corresponding Author:
S Subapriya
Assistant Professor, Centralized Clinical Laboratory, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, India

Gross, cytological, histopathological and Ultrastructural features of canine sweat gland adenocarcinoma: A case report

S Subapriya, N Pazhanivel, S Ramesh, Mohammed Ali, D Sumathi, M Sandhya Bhavani, Reeta Bascal and S Vairamuthu

Abstract
A 12 year old, male, Spitz breed dog was presented with a mass in the thigh region for tumour diagnosis. Samples were collected by Fine Needle Aspiration Biopsy (FNAB) for cytological examination. Tissue samples were collected post surgical excision for histopathological, immunohistochemical and ultrastructural investigation. Cytology of the mass revealed pleomorphic neoplastic cells containing round to oval nuclei and prominent multiple nucleoli. Histopathological examination revealed varying sized ducts and tubules containing pleomorphic neoplastic cells containing round to oval vesicular nuclei, prominent nucleoli and pale eosinophilic cytoplasm. Transmission Electron Microscopy (TEM) revealed tumour cells with features of anisokaryosis, multiple nuclei in a cell, margination of heterochromatin and prominent nucleoli with coarse chromatin. Based on the above findings the mass was diagnosed as sweat gland adenocarcinoma.

Keywords: Dog, sweat gland adenocarcinoma, apocrine tumour, apocrine adenocarcinoma, cytology, histopathology, Transmission Electron Microscopy (TEM), ultrastructural study

Introduction
Sweat gland tumours constitute 0.7-2.2 per cent of canine skin related tumours and they are generally encountered in inguinal and axillary regions (Goldschmidt and Hendrick, 2002) [1]. They occur in benign and malignant forms and have numerous histopathologic classifications, including cystadenoma, glandular adenoma, ductular adenoma and a variety of carcinoma subtypes (solitary, papillary, tubular, glandular, ductular, clear cell and signet ring (Cowell *et al*., 2007) [2]. Of the two types of sweat glands present in dogs, tumours are more frequently encountered in the apocrine sweat glands than in the eccrine sweat glands. Apocrine sweat gland tumours are relatively uncommon in dogs (overall 1.1% of all skin tumours) and perhaps a bit more common in cats (Hauck, 2013) [3]. Goldschmidt and Shofer (1992) [4] stated that approximately 70% of canine apocrine tumours are benign in nature, but malignant ones tend to recur locally and metastasize to regional lymph nodes and the lung.
Available literature on canine sweat gland adenocarcinoma is limited to the gross, cytological and histopathological findings and reports pertaining to the ultrastructural findings in sweat gland adenocarcinoma are of acute dearth. Hence, we place on record the ultrastructural findings observed in a case of canine sweat gland adenocarcinoma along with the cytohistopathological changes.

Materials and Methods
A twelve years old, male, Spitz breed dog was presented to the Small Animal Surgery-Out Patient ward and Small Animal Operation theatre - Surgery, of Madras Veterinary College Teaching Hospital (MVCTH), Chennai. The mass was grossly examined for its shape, size and location. Fine needle aspirates were collected for cytological examination. Tissue samples from the mass were collected after surgical excision for histopathological and ultrastructural examination. Blood sample for haematological examination was collected in EDTA vacutainer and processed in Auto haematology analyser. Serum sample for biochemical investigation was collected in clot activator tubes and processed in serum auto biochemical analyser.
For ultrastructural studies, tissues from excised mass were collected and fixed in 2.5 per cent gluteraldehyde and postfixed in phosphate-buffered 1 per cent osmium tetroxide and embedded in epoxy resin.

Sections from resection margins and adjacent normal areas in the wide excision specimens were taken as controls for comparison for ultrastructural comparison of tumour tissue and healthy skin. Ultrathin sections of 40 -60 nm were prepared using ultra microtome and were stained with aqueous saturated solution of uranyl acetate and lead citrate. Contrasted sections on the copper grid were then examined under Transmission electron microscopy (Tecnai 12G2 Biotwin) (120KV) in Wellcome Laboratory, Christian Medical College, Vellore, Tamil Nadu.

Results and Discussion

Gross examination of the tumour

Grossly, the mass appeared as an irregularly spherical hard mass measuring around 4 cm in diameter in the right thigh region (Fig.1). Nibe *et al.* (2005) [5] also reported that apocrine sweat gland tumours tend to occur on the head, neck, and limb in dogs. The mass was firm to hard on palpation. The skin overlying the tumour suspected mass was intact with no ulceration, hairloss or pruritus. The cut surface appeared light white to pink colour with cauliflower like multiloblations. The dog presented with the mass was 12 years old. Kalaher *et al.* (1990) [6] also reported that apocrine sweat gland tumours tend to occur in dogs from six to 17 years of age.

Cytological examination

Cytological examination revealed clusters of pleomorphic neoplastic cells (Fig.2) with round to oval nuclei containing prominent multiple nucleoli. The cytoplasm was eosinophilic in nature. This was in agreement with the findings of Cowell *et al*. (2007) [2a].

Histopathological examination

Histopathological examination revealed varying sized ducts and tubules containing pleomorphic neoplastic cells, single to multilayered, cuboidal to columnar containing basoeosinophilic to eosinophilic secretion, round to oval vesicular nuclei (Fig.3) and prominent nucleoli. Mitotic figures were also seen (Fig.4). The cytoplasm was pale eosinophilic. The histological findings were similar to the observations of Vasudevan *et al.* (2004) [7].

Ultrastructural findings

Transmission Electron Microscopic of the present case of sweat gland adenocarcinoma showed tumour cells with features of anisocytosis and anisokaryosis (Fig.5), multiple nuclei in a cell (Fig.6), margination of heterochromatin and atypical nuclei with prominent nucleoli containing coarse chromatin (Fig.7). The nuclear to nucleolar ratio was altered. Numerous mitochondria were found to be distributed in cytoplasm along with lipid vacuoles, rough endoplasmic reticulum, Golgi bodies, secretory vesicles and multivesicular bodies. Disrupted microvilli were seen as projections of outer surface of the cell. Kurosumi *et al.* (1984) [8] reported multivesicular bodies are a unique morphological feature of apocrine sweat glands of the dog and they have not been described in other mammalian species.

Blood vessels showed tumour emboli of neoplastic cells (Fig.8), in the lumen along with anisocytosis and poikilocytosis of erythrocytes which indicated the haematogenous spread of neoplasm in sweat gland adenocarcinoma. This is in agreement with the findings of Simko *et al.* (2003) [9] who stated that intravascular invasion is an important indicator of potential systemic metastases of apocrine gland adenocarcinoma in dogs. However, Baharak *et al.* (2012) [10] have claimed tumour metastasis due to lymphatic invasion in a case of apocrine sweat gland adenocarcinoma in a terrier dog because of the involvement of mediastinal lymph node.

The ultrastructural details observed in the present study suggesting the malignant nature of the tumour were in agreement with Cheville (1994)[11] who stated large and variable nuclei, large and irregular nucleoli and varied distribution of ribosomes, rough endoplasmic reticulum and lipid globules as the features of malignancy in a neoplastic cell at ultrastructural level. Pooling the ultrastructural findings with gross, cytological and histopathological features, the mass was confirmed as apocrine gland adenocarcinoma which is the malignant tumour of the apocrine sweat gland.

Haematobiochemical findings

The dog showed no significant changes in haematological and biochemical parameters studied.

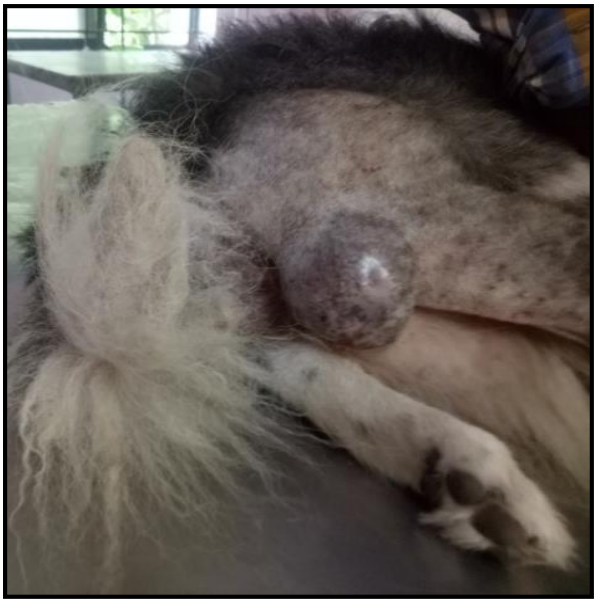

Fig 1: Sweat gland adenocarcinoma – Spitz- Right thigh – Spherical hard mass

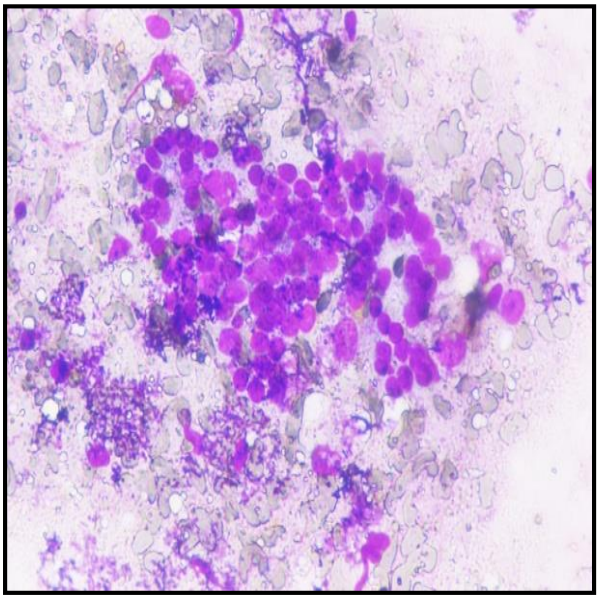

Fig 2: Sweat gland adenocarcinoma - Cluster of pleomorphic neoplastic cells LG x 400

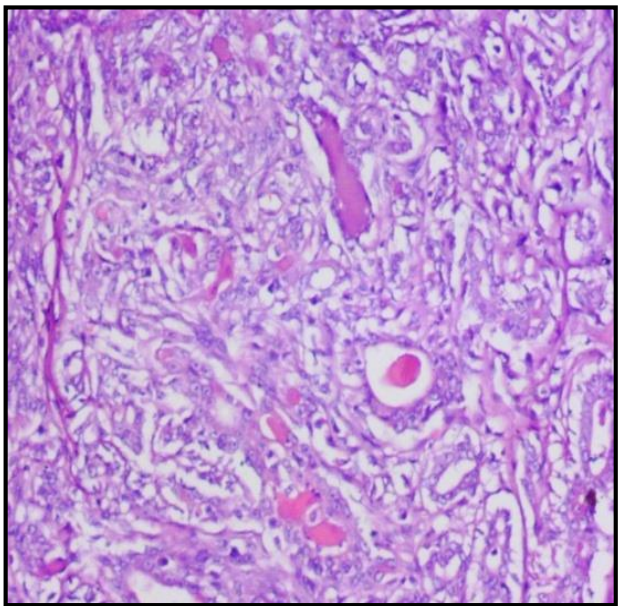

Fig 3: Sweat gland adenocarcinoma - Pleomorphic neoplastic cells with vesicular nuclei H&E x100

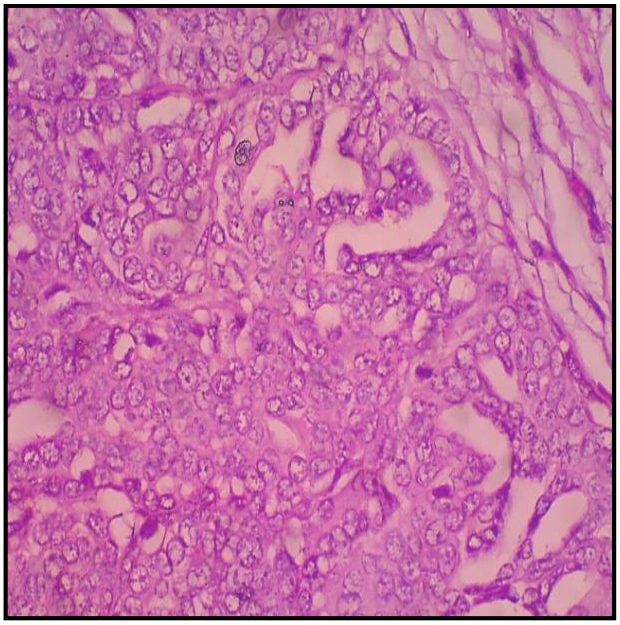

Fig 4: Sweat gland adenocarcinoma - Mitotic figures H&E x 400

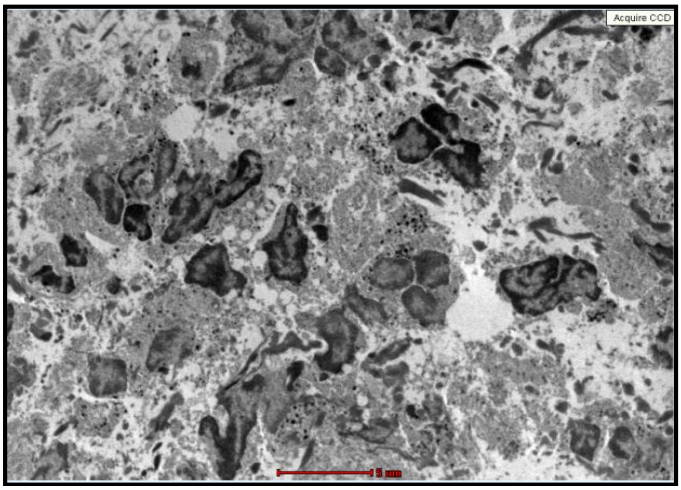

Fig 5: Electron micrograph – Sweat Gland Adenocarcinoma – Anisokaryosis -Multiple nuclei- Binucleated to multinucleated cells –Margination of heterochromatin- Uranyl acetate-Lead citrate x 2550

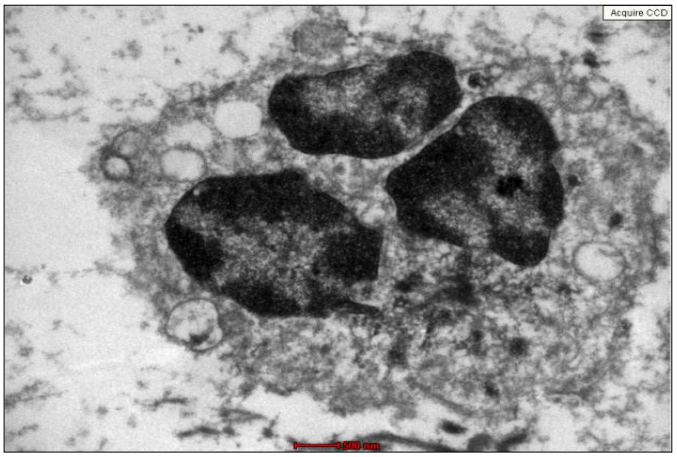

Fig 6: Electron micrograph – Sweat Gland Adenocarcinoma - Trinucleated cell- Margination of heterochromatin – Disrupted and protruding microvilli - Uranyl acetate-Lead citrate x 11500

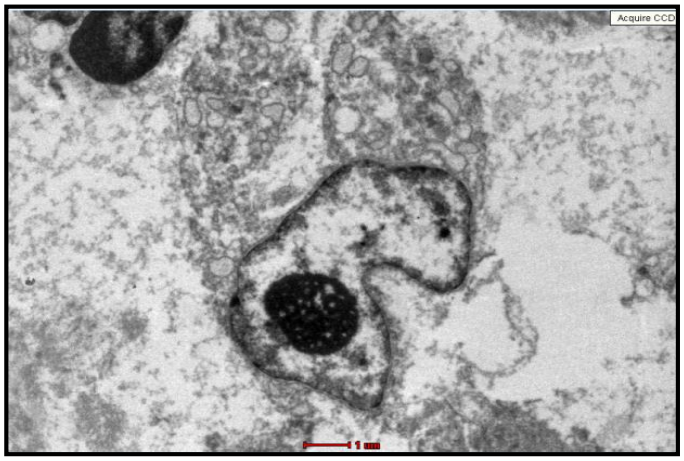

Fig 7: Electron micrograph – Sweat Gland Adenocarcinoma - Nuclear indentation- Prominent nucleoli – Coarse chromatin - Uranyl acetate-Lead citrate x 6000

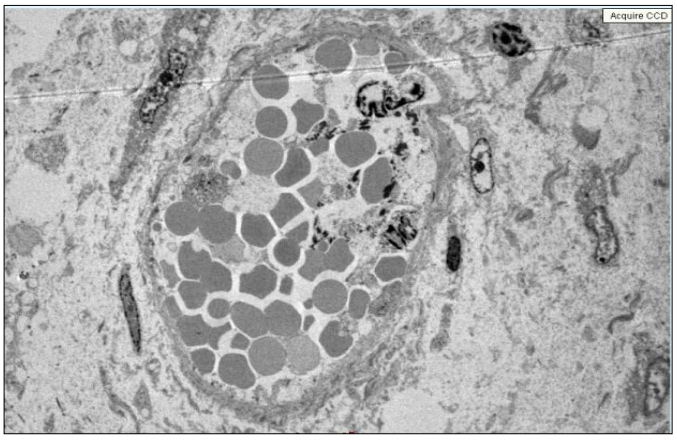

Fig 8: Electron micrograph – Sweat Gland Adenocarcinoma - Tumour Emboli - Uranyl acetate-Lead citrate x 1250

Conclusion

Based on the gross, cytological and histopathological findings, the tumour mass in thigh region of a spitz dog was diagnosed as sweat gland adenocarcinoma. The malignant nature of the tumour was further established by observing the morphological features of malignancy like anisocytosis, anisokaryosis, prominent nucleoli and increased distribution of secretory cells and organelles in ultrastructural sections studied with Transmission electron microscope.

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Conflicts of Interest
There is no conflict of interest.

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