

Squamous cell carcinoma in a dog - A case report

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ABSTRACT

A 12-year-old male Labrador was presented at a local multispecialty hospital in Chennai with a history of having a multinodular, ulcerated, greyish-white mass on the right forelimb near the digits for surgical removal. Various samples, including blood samples, peripheral blood smears, fine needle aspiration smears of the mass, and the excised mass were collected and sent to the Centralized Clinical Laboratory at Madras Veterinary College in Chennai for laboratory diagnosis. Hematological studies revealed anemia and leukocytosis with left shift neutrophilia, while no changes were observed in serum biochemical parameters. Cytological findings showed clusters of large neoplastic squamous epithelial cells with faintly basophilic cytoplasm, containing prominent vesicular single to multiple nuclei and nucleoli. Additionally, anisocytosis, anisokaryosis, mitotic figures and tadpole cells were suggested, indicative of squamous cell carcinoma. Histopathological examination revealed neoplastic cells that were polygonal in shape, containing prominent vesicular nuclei, mitotic figures and variable-sized keratin pearls. Moreover, immunohistochemical studies showed increased expression of vascular endothelial growth factor (VEGF) in the cytoplasm of the neoplastic cells, confirming the tumor as squamous cell carcinoma. Based on the current laboratory findings, the tumor was identified as well-differentiated squamous cell carcinoma.

Keywords: Cytology, dog, haematobiochemical, histopathology, squamous cell carcinoma

Squamous cell carcinoma (SCC), the most prevalent malignant neoplasm affecting dogs accounts for 5% of all cutaneous tumours. Although the dogs that are aged more than eight years are commonly affected, dogs that are four months age are also affected¹. The common sites of this tumour include nail bed, scrotum, nasal planum, limbs and anus. Squamous cell carcinoma affecting digits has been commonly recorded in large breeds, namely Standard Poodles, Labrador Retrievers, Giant Schnauzers, Gordon Setters, Rottweilers, Beaucerons and Briards. The etiology of squamous cell carcinoma is multifactorial. Exposure to solar ultraviolet light, ionizing radiation and chemical carcinogens, long-standing dermatoses, scars and other chronic lesions predispose the animals to this malignancy¹. The papilloma viruses causing immunosuppression and inducing malignant transformation have been found to be a potential risk factor for the development of this malignant neoplasm². Though the tumour can be extremely invasive and destructive, metastases are rarely recorded and occur only during the later stages of the disease. The common sites for metastasis include regional lymph nodes and lungs³. Various signs, namely scaly red spots, open sores and raised growths have been noticed in patients affected with squamous cell carcinoma⁴.

Based on differentiation, squamous cell carcinoma can be classified into well-differentiated, moderately differentiated and poorly differentiated. The well-differentiated subtype, which has been commonly recorded in canines, is characterized by masses or cords of neoplastic epithelial cells which proliferate and invade the dermis and subcutis. The hallmark of this tumour is the presence of keratin pearls in varying numbers and sizes, composed by concentric layers of squamous cells with a gradual increase of keratinization toward the center³.

A 12-year-old male Labrador dog was presented at a local multispecialty hospital, Chennai with the history of having a multinodular, ulcerated, greyish white mass on the forelimb near the digits for eight months, for surgical removal.

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Samples, namely EDTA added blood samples, clotted blood and peripheral blood smears, were collected and sent to the Centralised Clinical Laboratory, Madras Veterinary College, Chennai for haematobiochemical studies. In addition, fine needle aspiration smears from the mass were also collected and referred for cytological diagnosis. Based on the cytological diagnosis, surgery was performed a week later, and the excised mass was fixed in 10% formalin for histological studies.

Haematological and biochemical estimations were carried out using the Hemo auto analyzer namely Mindray BC-2800 and A15 Biosystems, respectively. The Fine Needle Aspiration Cytology (FNAC) smears as well as blood

smears were air dried, stained with Leishman and Giemsa cocktail stain and subjected to microscopical examination. Formalin-fixed tissues were processed by the routine paraffin embedding method, and the cut paraffin sections were stained by haematoxylin and eosin for histological studies. Immunohistochemical staining was performed on surgically excised tumour samples following the manufacturer protocol (M/s Biogenex, USA) using super-sensitive labeled poly-HRP polymer method.

Haemogram showed anemia and leucocytosis with left-shift neutrophilia while serum biochemical parameters revealed no abnormal changes. The haemato-biochemical profiles of the case are presented in Table 1.

Cytological findings revealed the presence of clusters of large neoplastic squamous epithelial cells with faintly basophilic cytoplasm containing prominent vesicular single to multiple nuclei and nucleoli. In addition, anisocytosis, anisokaryosis, mitotic figures and tadpole cells were also observed, which were suggestive of

squamous cell carcinoma (Fig. 1 & 2).

Histopathological examination revealed neoplastic cells that were polyhedral in shape containing a moderate amount of cytoplasm with distinct cell borders. The nuclei were round to oval and vesiculate, containing 1-3 prominent nucleoli with moderate anisokaryosis.

Table 1. Haematobiochemical parameters of the dog affected with Squamous cell carcinoma.

S.No.	Parameters	Values	Reference range
1.	Haemoglobin (g/dl)	7.00	12-18
2.	PCV (%)	24	35-55
3.	RBC (millions/cmm)	3.6	5.7-8.5
4.	WBC ($\times 10^3$ /cmm)	18	5-10
5.	Platelets ($\times 10^5$ /cmm)	2.6	1.5-3.5
6.	Differential count		
	i. Neutrophils	84%	60-70%
	ii. Lymphocytes	12%	12-28%
	iii. Eosinophils	4%	5-10%
7.	BUN (mg/dl)	12	10-28
8.	Creatinine (mg/dl)	1.02	0.5 to 1.5
9.	SGPT (ALT) (IU/L)	38	21-102
10.	ALP (IU/L)	106	20-156
11.	Total protein (g/L)	6.4	5.4-7.1
12.	Albumin (g/L)	3.2	2.3-3.8

In addition, mitotic figures and variable-sized keratin pearls, which were composed of concentric layers of squamous cells were noticed, confirming the mass as well-differentiated squamous cell carcinoma (Fig. 3). Immunohistochemical studies showed an increased expression of VEGF in the cytoplasm of the neoplastic

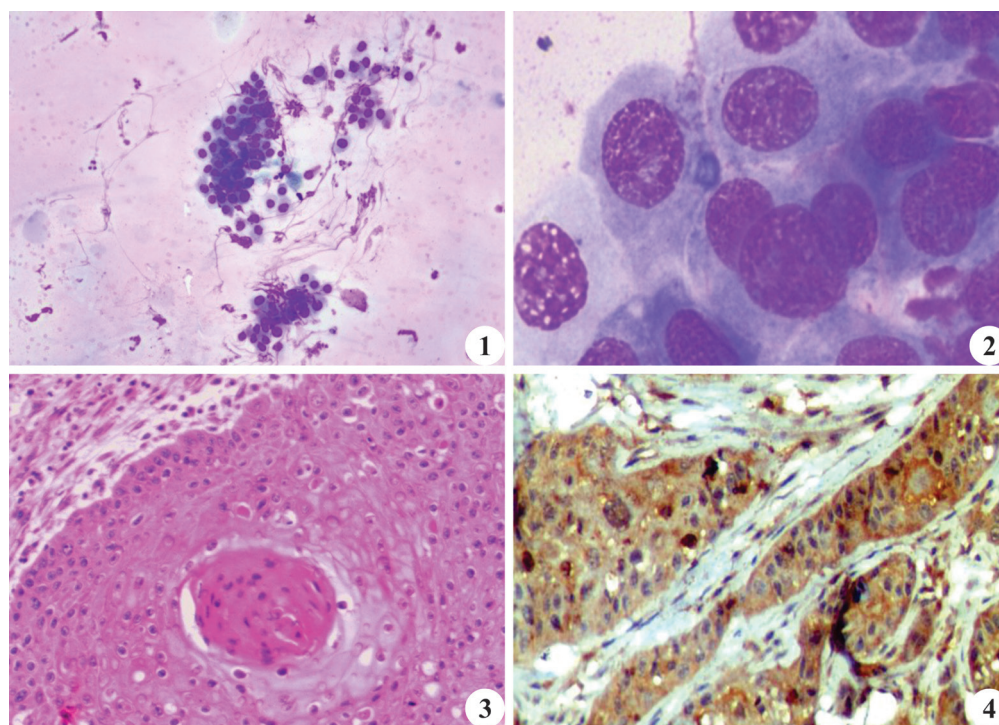


Fig. 1. Dog-Cytology: Squamous cell carcinoma - Clusters of neoplastic squamous epithelial cells showing anisocytosis and anisokaryosis (LG stain X100); **Fig. 2.** Dog-Cytology: Squamous cell carcinoma showing large neoplastic squamous epithelial cells showing prominent vesicular multiple nuclei and nucleoli with faintly basophilic cytoplasm (LG stain X100); **Fig. 3.** Dog-HP: Squamous cell carcinoma showing keratin pearls, composed of concentric layers of squamous cells (H&E X100); **Fig. 4.** IHC: Increased expression of VEGF in the cytoplasm of neoplastic squamous cells (X200).

cells which were suggestive of squamous cell carcinoma (Fig. 4). Based on the present laboratory findings, the tumour was identified as diagnosed as squamous cell carcinoma.

The present haematological findings were in accordance with those of earlier workers¹ who also reported leukocytosis and mild anemia in a 14-year-old dog affected with keratinized squamous cell carcinoma with extensive cranial bone invasion. An increased leukocyte count with normal biochemical findings was noticed in a 16-year-old female Maltese dog affected with squamous cell carcinoma⁵ as observed in the present study.

The cytological observations made in the present study correlate well with those of previous workers⁶ who also noticed similar cytological changes in dogs affected with squamous cell carcinoma. They observed a large number of malignant squamous cells occurring either individually or in clusters. The cells were pleomorphic, round to caudate in shape, exhibiting prominent anisokaryosis and anisocytosis. Anisokaryosis is characterised by nuclei, varying from pyknotic to large type, variable nuclear to cytoplasmic ratio, binucleation and multinucleation and perinuclear vacuolation. Similar cytological findings were also reported in domestic animals, including dogs, cats, horses and cows affected with squamous cell carcinoma⁷. The tadpole cells observed in the present study were also reported by earlier workers⁷ who opined that the presence of tadpole cells is not a diagnostic feature of squamous cell carcinoma but it actually indicates epithelial dysplasia, although it is suggestive of squamous cell carcinoma⁷.

The present histological findings agreed with that of previous workers^{8,9,10,12} who also recorded similar findings in domestic animals affected with squamous cell carcinoma. They found that neoplastic cells were polyhedral in shape containing moderate amounts of cytoplasm with prominent intercellular bridges. Nuclei were found to be round to oval and finely stippled to vesiculated, containing multiple prominent nucleoli. In addition, moderate to marked anisokaryosis, numerous mitotic figures and keratin pearls were noticed. The positive expression of cytokeratin in the cytoplasm of neoplastic cells recorded during the present study was

also reported by previous workers¹⁰ who noticed similar expression of cytokeratin AE1/AE3 in the cytoplasm of the neoplastic cells of a six-year-old male Pit Bull dog affected with squamous cell carcinoma.

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