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Histopathological and Immunohistochemical evaluations of canine cutaneous epithelial tumors

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Abstract
The present study was conducted to investigate the histopathological findings and immunohistochemical expression of Cytokeratin, Proliferating Cell Nuclear Antigen in canine cutaneous epithelial tumors. In this study, two cases each of Squamous Cell Carcinoma, Hepatoid gland adenoma, Sebaceous gland carcinomas and 3 cases of Basal Cell Carcinoma, were included. Strong to moderate expression of Intracytoplasmic immunoreactivity for Cytokeratin observed in all cases and Strong nuclear immunoreactivity for proliferating cell nuclear antigen observed in Poorly differentiated Squamous Cell Carcinoma compared to well differentiated Squamous Cell Carcinoma. Strong nuclear immunoreactivity for Proliferating Cell Nuclear Antigen observed in Basal Cell Carcinoma and sebaceous gland carcinoma. In heapatoid gland adenoma moderate Nuclear immunoreactivity for proliferating cell nuclear antigen observed. In this study, besides histopathology, effective systematic use of Cytokeratin, Proliferating Cell Nuclear Antigen confirmed the epithelial origin of tumors and helps in evaluating the proliferation rate.

Keywords: Cytokeratin, epithelial tumors, histopathology, immunohistochemistry, PCNA

1. Introduction
In pet veterinary medicine, the annually increasing incidence of malignant tumors is of great concern (Grüntzig *et al.*, 2015) [4]. In particular, dogs develop tumors at an incidence of more than 1,000 per 100,000 dog per year (Baioni *et al.*, 2017) [1], which is a higher rate than in humans. The incidence of cutaneous tumours in dogs is estimated to be 728 cases every year per 100, 000 dogs (Kaldrymidou *et al*., 2002) [6]. The demonstration of clinicopathological factors and biological markers with prognostic significance has become one of the most important fields of study in medical and veterinary oncology. These factors and markers are used to discriminate benign from malignant tumours, according to aggressiveness and metastatic risk, and to predict the prognosis (Mukaratirwa. 2005) [7]. Cytokeratin AE1/ AE3 is a mixture of two different clones of anti- cytokeratins monoclonal antibody. By combination of these two reagents, a single reagent with a broad spectrum of reactivity against both high and low molecular weight cytokeratins is obtained. (Vianne *et al*., 1995) [11]. Proliferating cell nuclear antigen (PCNA) is a 36-kDa nuclear protein that has been found to be a useful marker of cell proliferation since its expression and distribution are correlated with the rate of cell proliferation and DNA synthesis in various tumors (Yu CC and Filipe, 1933; Hall *et al*., 1990) [12, 5]. PCNA increases after the G1 phase of the cell cycle, reaching a maximum at the S phase and decreasing after G2; then, it presents low levels in the M phase and quiescent cells (Tsuji T *et al*., 1992) [10]. The present paper communicates the histopathological findings and immunohistochemical expression of Cytokeratin (AE1/AE3), PCNA (proliferating cell nuclear antigen) in cutaneous epithelial tumors of Dog.

2. Materials and Methods
In this study, two cases of Squamous Cell Carcinoma (SQCC), 3 cases of Basal Cell Carcinoma (BCC), two cases of Hepatoid gland adenoma and two cases of Sebaceous gland carcinomas were included for histopathology and immunohistochemistry. The tissue samples were processed by paraffin-embedding technique. Sections of 4 µm were obtained and stained with routine Haematoxylin and Eosin staining method. Immunohistochemistry was performed with mouse monoclonal primary antibody to Cytokeratin (AE1/AE3), PCNA (proliferating cell nuclear antigen) by using Super-sensitive Polymer-HRP detection kit. 4µ thickness sections of lung tissue sections were deparaffinised, rehydrated and transferred to citrate buffer placed in a microwave oven (800 W) for 30 min for antigen retrieval and incubated for 10 min in Peroxide

bock TM and Power bock TM, Primary antibody Cytokeratin applied to the sections and incubated for 60 min at room temperature. The sections were rinsed in TBS and Super enhancer TM was added to slides incubated for 20 min. Then the sections were rinsed in TBS buffer and were covered with Poly-HRP reagent for 10 min. Sections were rinsed in TBS buffer. The sections were then incubated with solution prepared by adding one drop of liquid DAB chromogen mixed with 1ml of buffer for 10 min. Sections were rinsed with TBS, washed with distilled water, counter stained with Harris Haematoxylin and mounted with DPX mountant. Scoring of Cytokeratin was done based on the percentage of positive cells i.e., 0: no immunostaining, 1: 1-25%, 2: 26-50%, 3:51-75% and 4: 76-100% positive stained cells. Scoring of PCNA was done based on the percentage of positive cells i.e., 0: <5%, 1: 5-25%, 2: 26-50%, 3:51-75% and 4: >75% positive stained cells. The scoring of Cytokeratin and PCNA was done as per the method described earlier[5].

Results and Discussion

Microscopic examination revealed islands of neoplastic squamous cells with keratin pearl in well differentiated SQCC and revealed islands of neoplastic cells devoid of keratin pearls in poorly differentiated squamous cell carcinoma. The proliferating neoplastic cells revealed moderate cellular pleomorphism, large vesicular nuclei, prominent nucleoli and variable mitotic activity observed in poorly differentiated SQCC (Fig-1). BCC revealed nests of basal cells with peripheral palisading arrangement. These nests are separated from the surrounding stroma by cleft like space (Fig-4). These histopathological features of SQCC and BCC were similar to the findings of earlier reports [8]. Hepatoid gland adenomas revealed the presence of polygonal neoplastic cells arranged in sheets with small basophilic reserve cell at the periphery. Presence of round to oval nucleus and moderate amount of eosinophilic cytoplasm resembling hepatocytes. (Fig-7). These histopathological features were in consistent with findings of Chikankar et al., (2022) [2]. Sebaceous gland carcinomas characterized by irregular lobular formation with a variable degree of sebaceous differentiation. The lobules were separated by connective tissue proliferation. Some neoplastic cells revealed vacuolated cytoplasm and large vesicular nuclei with indistinct boundaries (Fig-10). Histopathological features of sebaceous gland carcinoma are in consistent with the findings of Costa *et al*., (2020) [3].

In this study Strong expression of Intracytoplasmic Immunoreactivity for Cytokeratin (AE1/AE3) was noticed in well differentiated SQCC and BCC (Fig- 5), moderate expression of cytokeratin was observed in poorly differentiated SQCC as well developed keratinization is not *seen in poorly differentiated SQCC* (Fig-2), hepatoid gland adenoma and sebaceous gland carcinoma also exhibited moderate cytoplasmic innumoreactivity for Cytokeratin (Fig-8,11) and Strong Nuclear immunoreactivity for PCNA observed in poorly differentiated SQCC compared to well differentiated SQCC (Fig-3) indicating the high proliferating ability of Poorly differentiated SQCC which in turn indicates aggressive nature of the tumor. Strong nuclear immunoreactivity for PCNA observed in basal cell carcinoma and sebaceous gland carcinoma (Fig-6, 12). In hepatoid gland adenoma moderate nuclear immunoreactivity for PCNA (Fig-9) observed. Increase in PCNA score indicates Cell proliferation significantly contributed to the degree of malignancy where the less differentiated cells showed a stronger ability to proliferate, thus enhancing tumor malignancy. These histopathological and immunohistochemical finding were in accordance with the previous report of Paramjeet *et al*., (2021) [8]. Paramjeet *et al*., (2021) [8] stated that besides histopathology, immunohistochemistry by using tumor markers viz cytokeratin and PCNA ascertain the epithelial origin of tumor cells and PCNA for assessing the proliferation rate and prognosis of tumors. Immunohistochemical expression of PCNA in this study were in consistent with the findings of Subapriya *et al*., (2021) [9]. Authors opined that Immunohistochemical staining with PCNA revealed strong and diffuse positive expression of PCNA in Squamous cell carcinoma and Sebaceous gland adenocarcinomas indicating the proliferating ability of these tumours.

Table 1: Showing Immunoreactivity of cells in different tumors for Cytokeratin (AE1/AE3) and PCNA

S. No	Tumor	Cytokeratin (AE1/AE3) Score	PCNA Score
1	Well differentiated SQCC n=1	4	2
2	Poorly differentiated SQCC n=1	3	3
3	Basal cell carcinoma n=3	4	4
4	Hepatoid gland adenoma n=2	3	2
5	Sebaceous gland carcinoma n=2	3	4

In conclusion, following the human oncology approach for Immunohistochemistry to identify Cancer of unknown primary sites and to predict the prognosis, in canines also effective systematic use of Selective immunohistochemical markers based on the results of histopathology can be employed to enable the accurate diagnosis in early stages, in understanding the malignancy features to predict the prognosis.

Figures

Fig- 1 Poorly differentiated Squqmous cell carcinoma. showing nests of squamous cells. H&E 400X

Fig- 2 Poorly differentiated Squqmous cell carcinoma. Moderate Cytoplasmic expression of Cytokeratin in Squamous cells. IHC Cytokeratin 400x

Fig- 3 Poorly differentiated Squqmous cell carcinoma. Strong nuclear immunoreactivity IHC PCNA 400x

Fig - 4 Basal cell carcinoma. Showing basal cells arranged in nests with peripheral palisading arrangement. H&E 400X

Fig - 5 Basal cell carcinoma. Strong Cytoplasmic expression of Cytokeratin in Epithelial cells. IHC Cytokeratin 400x

Fig - 6 Basal cell carcinoma. Strong Positive nuclear immunoreactivity in proliferating cells IHC PCNA 400x

Fig -7 Hepatoid gland adenoma. Polygonal neoplastic cells arranged in sheets. H&E 400X

Fig - 8 Hepatoid gland adenoma. Moderate Cytoplasmic expression of Cytokeratin in Epithelial cells. IHC Cytokeratin 400x

Fig-9. Hepatoid gland adenoma. Moderate Positive nuclear immunoreactivity in proliferating cells IHC PCNA 400x

Fig - 10 Sebaceous gland carcinoma. Showing irregular lobular formation with a variable degree of sebaceous differentiation. H&E 400X.

Fig - 11 Sebaceous gland carcinoma. Moderate Cytoplasmic expression of Cytokeratin in Epithelial cells. IHC Cytokeratin 400x

Fig -12 Sebaceous gland carcinoma. Strong Positive nuclear immunoreactivity in proliferating cells. IHC PCNA 400x.

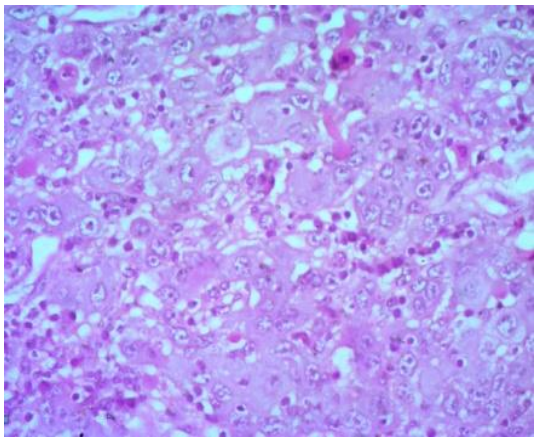

Fig 1: SQCC (H&E 400X)

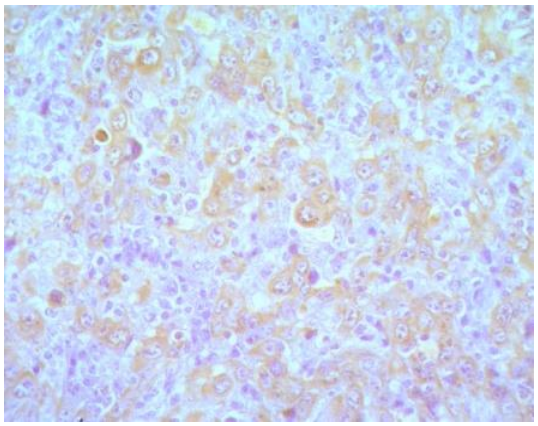

Fig 2: SQCC IHC Cytokeratin

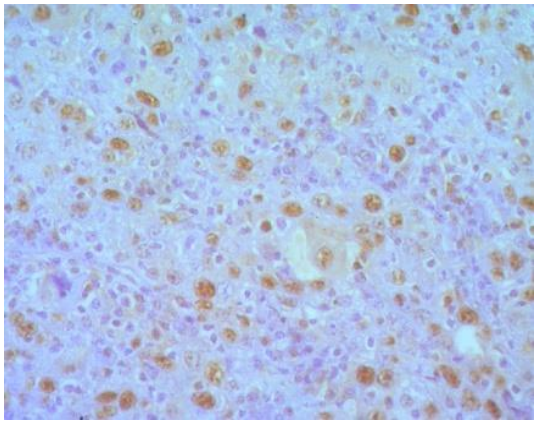

Fig 3: SQCC IHC PCNA 400x

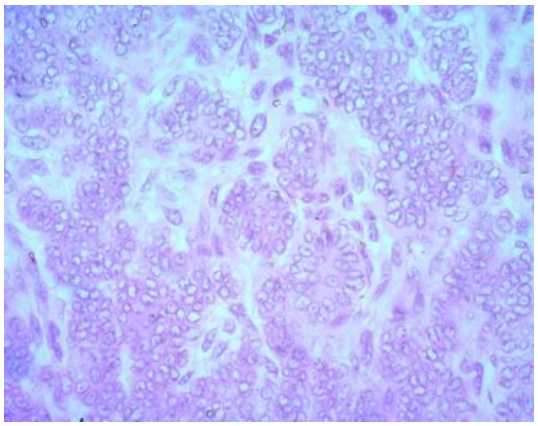

Fig 4: Basal cell carcinoma (H&E 400X)

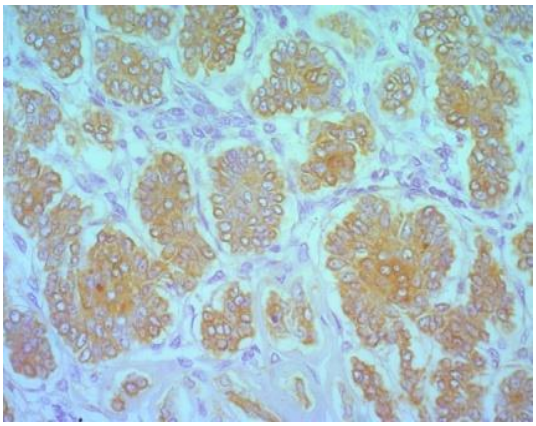

Fig 5: Basal cell carcinoma
IHC Cytokeratin 400x

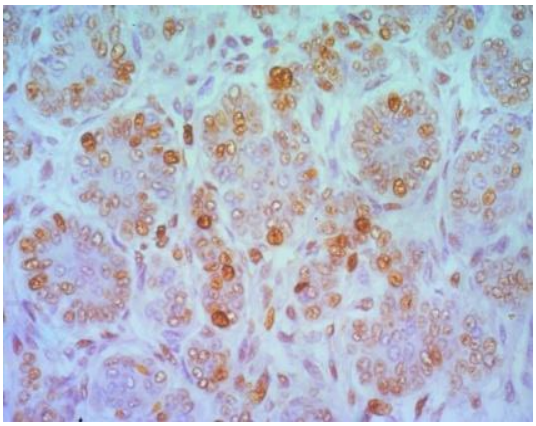

Fig 6: Basal cell carcinoma
IHC PCNA 400x

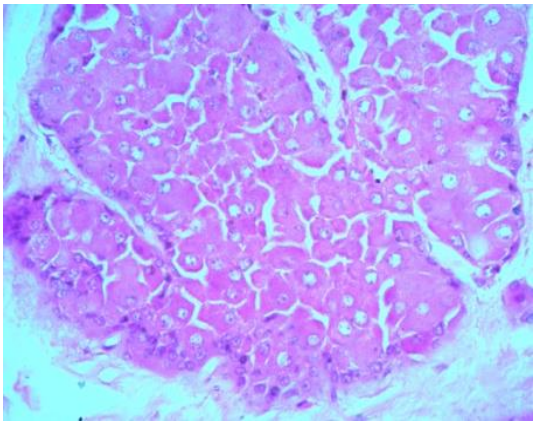

Fig 7: Hepatoid gland adenoma (H&E 400X)

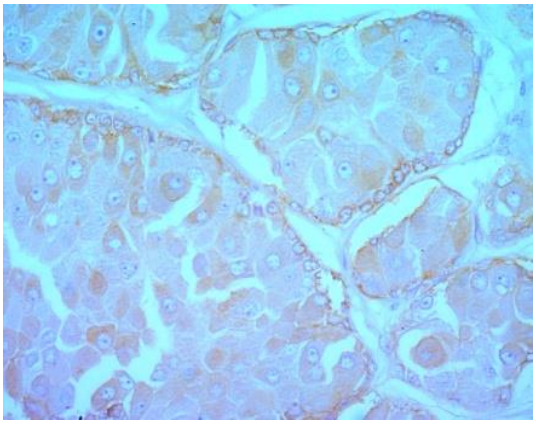

Fig 8: Hepatoid gland adenoma IHC Cytokeratin 400x

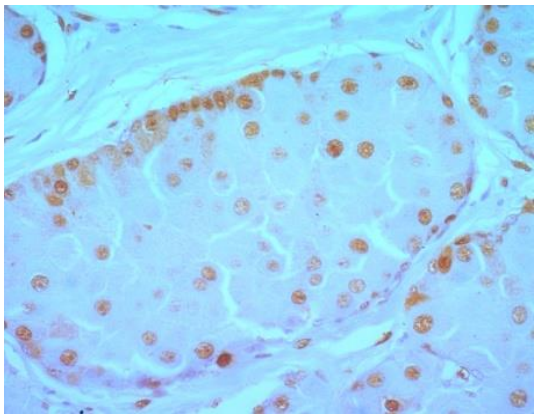

Fig 9: Hepatoid gland adenoma IHC PCNA 400x

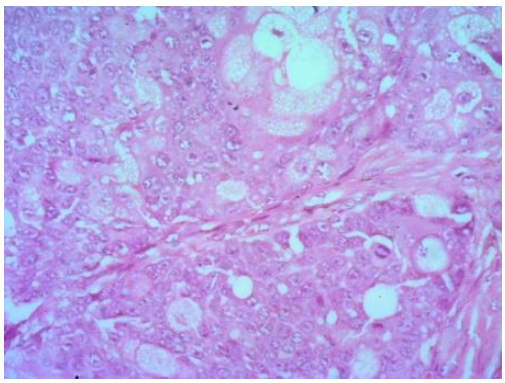

Fig 10: Sebaceous gland carcinoma (H&E 400X)

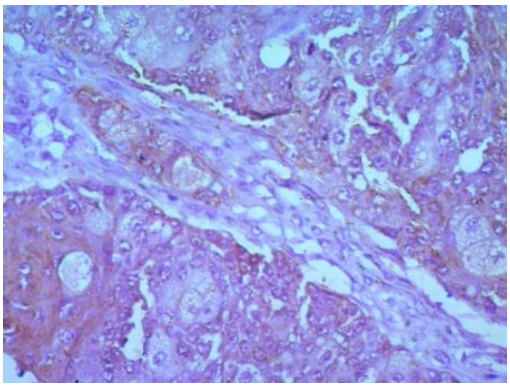

Fig 11: Sebaceous gland carcinoma IHC Cytokeratin 400x

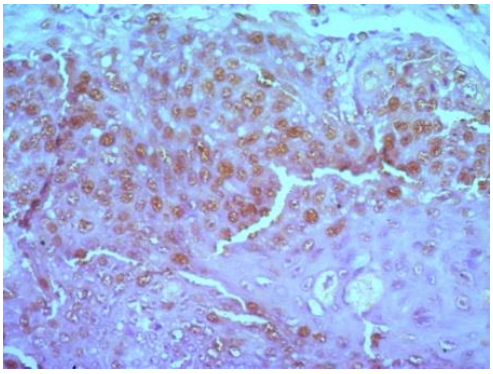

Fig 12: Sebaceous gland carcinoma IHC PCNA 400x

Reference

1. Baioni E, Scanziani E, Vincenti MC, Leschiera M, Bozzetta E, Pezzolato M, *et al*. Estimating canine cancer incidence: findings from a population-based tumour registry in Northwestern Italy. BMC Veterinary Research. 2017;13:203.
2. Chikankar RP, Patil AP, Rohi RR, Sawale GK, Dagli NR, Ingole KH. Perianal (Hepatoid) Gland Adenoma in Labrador Dog: Its Diagnosis and Surgical Management. International Journal of Livestock Research. 2022;12(7):1-13.
3. Costa F, Kássia S, Juliana L, Franciele S, Bárbara B, Juliana S, *et al*. Histopathological study of canine sebaceous tumors and their association with PCNA expression by immunohistochemistry. Revista Brasileira de Ciência Veterinária. 2020;27:150-158. 10.4322/rbcv.2020.027.
4. Grüntzig K, Graf R, Hässig M, Welle M, Meier D, Lot G, *et al*. The Swiss canine cancer registry: a retrospective study on the occurrence of tumours in dogs in Switzerland from 1955 to 2008. Journal of Comparative Pathology. 2015;152:161-171.
5. Hall PA, Levison DA, Woods AL, Yu CC, Kellock DB, Watkins JA, *et al*. Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. Journal of Pathology. 1990;162:285-294.
6. Kaldrymidou H, Leontides L, Koutinas AF, Saridomichelakis MN, *et al.* Prevalence, distribution and factors associated with the presence and the potential for malignancy of cutaneous neoplasms in 174 dogs admitted to a clinic in northern Greece. Journal of veterinary medicine A, Physiology, pathology, clinical medicine. 2002;49:87-91.
7. Mukaratirwa S. Prognostic and predictive markers in canine tumours: Rationale and relevance. A review, Veterinary Quarterly. 2005;27(2):52-64. DOI:10.1080/01652176.2005.9695186
8. Paramjeet, Babu Lal J, Sandeep S, Deepika L. Pathomorphological and Immunohistochemical evaluations of pancytokeratin, vimentin and proliferating cell nuclear antigen in epithelial tumours of dogs. Indian Journal of veterinary Pathology. 2021;45(2):128-135.
9. Subapriya S, Pazhanivel N, Shafiuzama M, Sumathi D, Jayanthy C, Vairamuthu S. Immunohistochemical diagnosis of skin tumours in dogs. The Pharma Innovation Journal. 2021;SP-10(5):612-619.
10. Tsuji T, Shrestha P, Yamada K, Takagi H, Shinozaki F, Sasaki K. Proliferating cell nuclear antigen in malignant and pre-malignant lesions of epithelial origin in the oral cavity and the skin: An immunohistochemical study. Virchows Archiv. A, Pathological anatomy and histopathology. 1992;420(5):377-383.
11. Vianne A, Baert J. Expression of cytokeratin-mRNAs in squamous cell carcinoma and balloon-cell formation of human oesophageal epithelium. The Histochemical Journal. 1995;27:69-78.
12. Yu CC, Filipe MI. Update on proliferation-associated antibodies applicable to formalin-fixed paraffin embedded tissue and their clinical applications. The Histochemical Journal. 1993;25:843-853.