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A STUDY OF HISTOPATHOLOGICAL VARIANTS OF SQUAMOUS CELL CARCINOMA IN DOGS

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ABSTRACT

Squamous cell carcinomas (SCCs) are malignant epithelial tumors arising from keratinocytes, exhibiting varying degrees of differentiation. They rank among the most prevalent cutaneous neoplasms in dogs. This study documents nine cases of SCC diagnosed in dogs at the Veterinary Clinical Complex, DUVASU Mathura, between 2023 and 2024. Grossly, the tumors presented as nodular or cauliflower-like masses, frequently ulcerated. Fine-needle aspiration cytology (FNAC) revealed highly pleomorphic cells with features including anisocytosis, karyomegaly, elevated nucleus-to-cytoplasmic ratio, binucleation, multinucleation, hyperchromasia and cytoplasmic basophilia. Histopathological examination classified the tumors into three distinct differentiation patterns: well-differentiated, moderately differentiated, and poorly differentiated SCC. The degree of histopathological differentiation serves as a critical prognostic indicator for these malignancies. Aberrant E-cadherin expression was consistently observed across all three histological grades of the tumor.

Keywords: squamous cell carcinoma, canine, e- cadherin, histological grading

INTRODUCTION

Squamous cell carcinoma (SCC) is one of the most frequently diagnosed tumors in dogs, with a mean age at diagnosis of approximately 8 years (Strafuss, 1976). Due to its nonspecific and heterogeneous gross presentation, confirmatory diagnosis

requires microscopic evaluation via histopathology or cytology (Withrow et al., 2012). Histological grading plays a crucial role in assessing the biological behavior of SCC. A multifactorial grading system for SCC has been previously described (Anneroth et al., 1987), and the degree of differentiation has been identified as an independent prognostic factor for overall survival in affected dogs (Brinkman et al., 2015).E-cadherin, an epithelial calcium-binding transmembrane glycoprotein, mediates cell-to-cell adhesion and is essential for maintaining stable intercellular junctions (van Roy & Berx, 2008). Altered or loss of membranous E-cadherin expression has been associated with tumor invasiveness in various cancers, including cervical squamous cell carcinoma (Vessey et al., 1995; Mahomed et al., 2007). Given its established role as a potential biomarker for SCC in previous studies (von Zeidler et al., 2014), E-cadherin was selected as a key marker for this investigation.This study aims to characterize the histopathological variants of canine squamous cell carcinoma and evaluate the immunohistochemical expression pattern of E-cadherin in neoplastic squamous epithelial cells.

MATERIALS AND METHODS

Sample collection and processing

A total of nine biopsy samples were obtained from nine different dogs diagnosed with squamous cell carcinoma (SCC) at the Veterinary Clinical Complex (VCC), DUVASU, Mathura, during the period 2023–24. All experimental protocols were conducted following approval by the Institutional Animal Ethics Committee (IAEC) of U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidhyalaya Evam Go-Anusandhan Sansthan, Mathura-281001 (Approval Nos. IAEC/21/24, dated 18.03.2022, and IAEC/22/19, dated 31.01.2021)

Fine-needle aspiration cytology (FNAC) of the masses was performed, and smears were routinely stained with Giemsa for cytological evaluation (Cowell et al., 2007). Surgically excised tissue samples were fixed in 10% buffered formalin and proceeded for routine histopathology. Staining was done with hematoxylin and eosin (H&E). Histological grading of the sections was conducted following established criteria from prior studies (Anneroth et al., 1987). According to the established classification system the various parameters such as keratinization, nuclear polymorphism, mitotic activity, extent of invasion and inflammatory infiltration — were assessed, with each parameter assigned a score ranging from 1 to 4. The total histologic score for each case was calculated as the sum of individual

parameter scores, with subsequent grading as follows: Grade I (Well-differentiated): Total score of 6–10; Grade II (Moderately differentiated): Total score of 11–15 & Grade III (Poorly differentiated): Total score of 16–20 (Cerezo-Echevarria et al., 2020).

Post-diagnostic and treatment follow-up data were collected throughout the study period via structured telephonic interviews with the owners.

Immunohistochemistry

For immunohistochemical analysis, 5-µm-thick tissue sections were mounted on APES-coated slides. Heat-induced epitope retrieval (HIER) was performed using 10 mM citrate buffer (pH 6.0) in a microwave (600 W, 25 min), followed by quenching endogenous peroxidase activity with 3% hydrogen peroxide (H_2O_2) for 10 minutes. Non-specific binding was blocked with 2% bovine serum albumin (BSA) for 10 minutes. The sections were then incubated overnight at 4°C with Mouse anti-canine anti- E cadherin primary antibody at a 1:20 dilution. After washing with PBS, the slides were treated with HRPO-conjugated anti-mouse IgG secondary antibodies (1:50 dilution) for 2 hours at room temperature. Immunoreactivity was visualized using 3, 3′-diaminobenzidine (DAB) as the chromogenic substrate.

RESULTS

Signalment:

Among the nine affected animals, the most frequently represented breeds were German Shepherd Dog (3/9), followed by Pomeranian (2/9). Cases were also observed in Labrador Retrievers, Golden Retrievers, Spitz, and Mongrels. The median age at presentation was 6 years, with a male predominance (77.78%) compared to females (22.22%). The most common anatomical sites for tumor occurrence included the oral cavity, dorsal region, forelimbs, and ocular tissues.

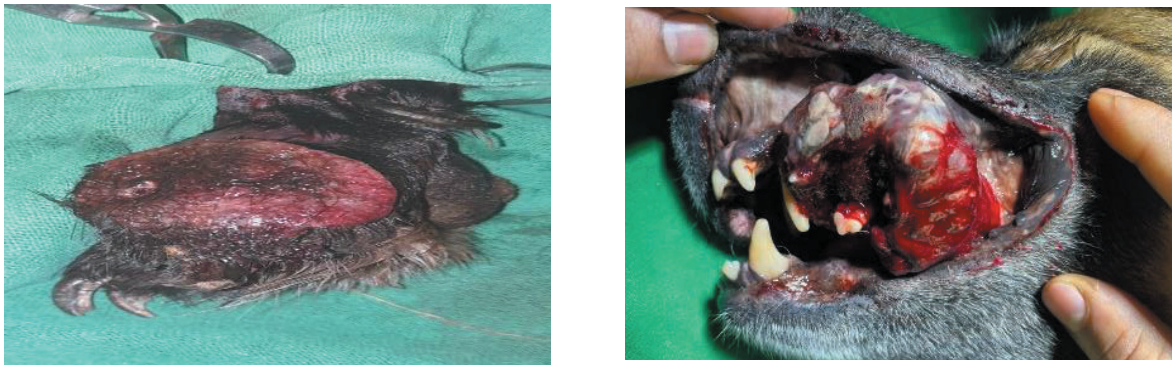

Fig. 1A. A large well circumscribed, ulcerated mass on the dorsal aspect of forelimb of a 3 year old GSD. B. Another cauliflower like mass on the maxilla of a 6 year old GSD.

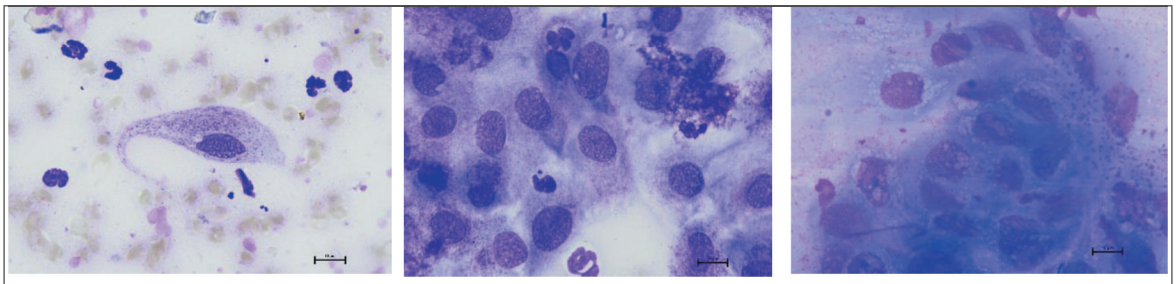

Fig. 2A. FNAC of the tumor sample showing a tadpole shaped cell indicative of SCC. **B.** These cells have increased nucleus to cytoplasmic ratios, cytoplasmic basophilia and hyperchromatic pleomorphic nuclei. **C.** Some of these cells are seen in whorls Giemsa 1000x.

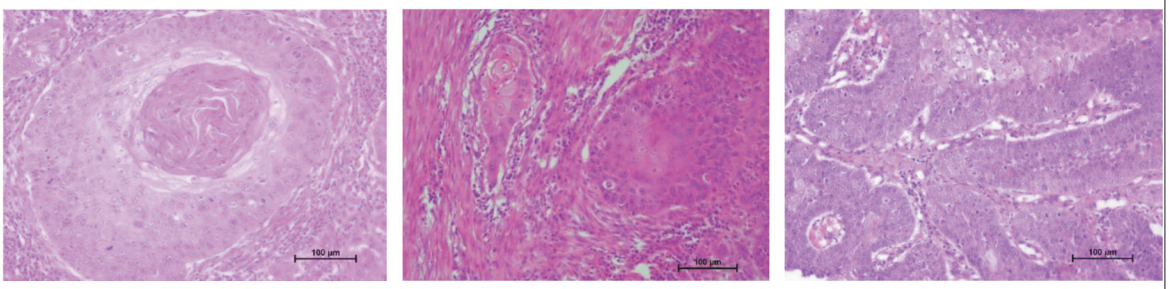

Fig. 3A. Histopathological section of a well differentiated grade I SCC showing epithelial pearls with marked keratinization a few mitotic figures (arrows). **B.** Moderately differentiated SCC showing moderate amount of keratinization and nuclear pleomorphism and moderate host inflammatory response (star) **C.** Poorly differentiated or undifferentiated SCC showing abundant mitotic figures and high nuclear pleomorphism with minimal host inflammatory response H&E 200x.

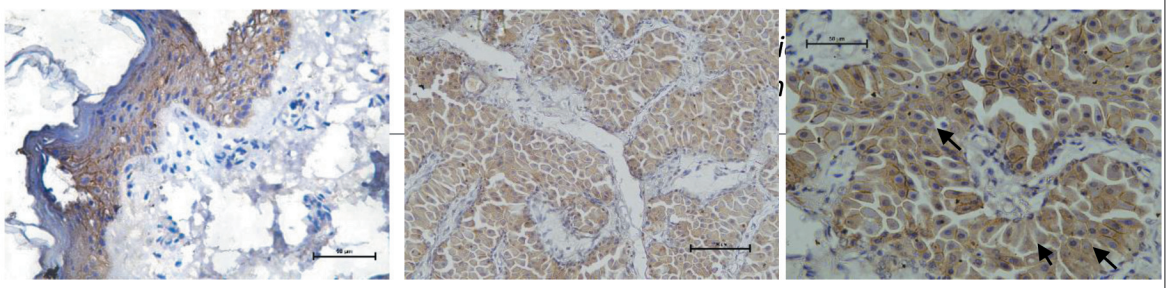

Fig. 4A. Immunohistochemistry of normal canine skin showing the membraneous staining pattern of E cadherin in the epidermis **B.** A few cells showing membraneous and rest showing aberrant cytoplasmic staining of E cadherin in moderately differentiated SCC H&E 200x **C.** Higher magnification showing loss of membraneous staining pattern and aberrant cytoplasmic immunostaining of E cadherin H&E 200x.

Grossly the SCCs appeared as nodular or cauliflower like with ulcerated surfaces Fig 1A. The fine-needle aspiration cytology (FNAC) of the tumor mass revealed large malignant squamous cells exhibiting an increased nuclear-to-cytoplasmic ratio, nuclear hyperchromasia, pleomorphism, basophilic cytoplasm, and tadpole-shaped cells. Fig. 2 A, B & C.

Histopathological examination of the tumor masses revealed three distinct variants of squamous cell carcinoma (SCC), consistent with previous classifications: well-differentiated SCC (04/09), moderately differentiated SCC (02/09), and poorly differentiated SCC

Grade (Total Score)	*Cases (n=9)*	*Prognostic outcome (within 12 month study period)*	
		Alive	Dead
Grade 1	4 (44.44%)	4	-
Grade 2	2(22.22%)	1	1
Grade 3	3(33.33%)	1	2

Table 1 : showing various histological grades of SCCs and their prognostic outcome in a 12 month study period.

(03/09). Well-differentiated squamous cell carcinomas are characterized by prominent keratinization, evidenced by epithelial pearl formation, along with minimal mitotic activity (Srivastav et al., 2022). Moderately differentiated SCCs exhibited moderate keratinization, nuclear pleomorphism, and a moderate host inflammatory response. In contrast, poorly differentiated or undifferentiated SCCs displayed abundant mitotic figures, marked nuclear pleomorphism, and a minimal host immune reaction (Fig. 3 A, B & C)

Immunohistochemical analysis revealed aberrant E-cadherin expression in the tumor samples, characterized by either loss of membranous staining or discontinuous expression patterns in all the three grades of tumours. Abnormal staining included fragmented or absent membrane localization, occasionally with or without cytoplasmic cytoplasmic staining. The normal canine epidermis showed membrane expression of E- cadherin in more than 75% of epidermal cells across the basal, spinous and granular layers. Fig. 4 A showing characteristic membranous E-cadherin expression, serving as a positive control for comparative analysis.

Table 1 presents the prognostic outcomes for all three histological grades of squamous cell carcinomas (SCCs) in dogs. Dogs with histological grade 2 and 3 SCCs exhibited a poorer prognosis compared to those with grade 1 tumors, which demonstrated a more favorable outcome. However, no significant differences in immunohistochemical staining patterns of E-cadherin were observed among the three grades.

DISCUSSION

Squamous cell carcinoma (SCC) in dogs has been reported to affect both males and females equally, demonstrating no sex predilection (Willcox et al., 2019). The tumor commonly occurs in regions such as the gums, back, perianal area, and lower abdomen, with the highest incidence observed between 6 to 9 years of age (Chandrashekeraiah et al., 2011). The gross morphological features of the tumor in this study were consistent with previous descriptions (Goldschmidt & Hendrick,

2002; Withrow et al., 2013).

Histologically, canine SCC has been classified into three distinct variants (Goldschmidt & Hendrick, 2002). Multiple studies have emphasized the prognostic significance of histological grading, with higher-grade tumors associated with poorer outcomes (Pereira et al., 2007; Larsen et al., 2009; Nemec et al., 2012).

Previous studies (Ressel et al., 2020), consistent with our observations, have reported diminished membranous expression and aberrant cytoplasmic localization of E-cadherin in canine squamous cell carcinomas. However, the present study did not identify significant variations in E-cadherin expression patterns among different histological grades, possibly due to the limited sample size.

Further investigations utilizing larger cohorts and additional molecular markers alongside E-cadherin are warranted to elucidate its potential role as a prognostic indicator in canine SCC.

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