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Clinical Article

HISTOPATHOLOGICAL AND CYTOPATHOLOGICAL FINDINGS OF CANINE SQUAMOUS CELL CARCINOMA

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SUMMARY

The study aimed to determine the histopathological and cytological findings which can effectively identify Squamous Cell Carcinoma (SCC). In the present investigation, 102 cases of tumor like growths were evaluated and 20 SCC were diagnosed based on cytological and histopathological observations. Cytological examination revealed the presence of large number of malignant pleomorphic squamous tumour cells arranged individually or in clumps showing anisocytosis and anisokaryosis. Histopathological examination of well differentiated SCC revealed neoplastic cells arranged in the form of irregular cords or whorls within the tumour, typical eosinophilic lamellated "epithelial pearl" or "keratin pearl" associated with focal to diffuse areas of necrosis and infiltration of inflammatory cells. Moderately differentiated SCC was characterized by whorls of proliferating neoplastic cells separated by thin fibrous stromal components with occasional cell nests and minimal keratinization. Poorly differentiated SCC showed indistinct epithelial layering and indistinct or absence of keratinization. The diagnosis of SCC will be helpful, if evaluated with cytological and histopathological findings of the related stage.

Keywords: Cytology, Histopathology, Squamous cell carcinoma

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Neoplasms in dogs are twice more frequent in comparison to man, which progress more rapidly and bear similar anatomical and physiological properties, proving them as an excellent animal model for understanding human cancers (Chandrashekaraiah *et al.*, 2011). Cancer is a consequence of imbalance between cell death and proliferation in a way favourable to cell proliferation and survival (Mobahat *et al.*, 2014). In past times, cancer has been reported to be the leading cause of mortality in dogs and second most in humans (Jemal *et al.*, 2008). Diagnosis of cancer is a crucial requirement to take up treatment, is accomplished mainly by microscopic examinations including histopathology and cytology. Squamous cell carcinoma (SCC) is a malignant tumour of stratified squamous epithelium. It is the common form of carcinoma occurring in all domestic species. The gross appearance of SCC can be variable and nonspecific, so definitive diagnosis requires microscopic examination of the tissue (Chandrashekaraiah *et al.*, 2011).

In dogs, less than 5% of cutaneous neoplasms are SCC, and common sites include the legs, scrotum, perineum, nasal planum, and various lightly pigmented areas (Meuten, 2017; Withrow and Vail, 2012). SCCs account for 70% of feline and 25% of canine oral neoplasms and may arise from virtually any surface in the oral cavity, including gingiva, tongue, tonsils, pharynx, lips, and buccal mucosa (Meuten, 2017). This study aimed to determine the histopathological and cytogical findings which can effectively identify squamous cell carcinoma.

This study was conducted in the Department of Veterinary Pathology, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam during 2020-2021, on 20 cases of cutaneous and subcutaneous tissue tumors of dogs presented to the Department of Veterinary Surgery and Teaching Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, India. Apart from visible skin masses, patients were declared as healthy based on pre-operative hematological and serological parameters. On owner's consent, surgery was carried out for cutaneous masses, and tissues were collected for histopathological processing. Breed of dogs encountered in the present study were Labrador (9), followed by German shepherd (5), Boxer (4) and Doberman (2).

Fine needle aspiration cytology (FNAC) was for cytological examination in order to confirm neoplastic condition. Impressions were taken from the excised tumour and smears were prepared and stained using standard Giemsa-Wright's technique (Luna, 1968). Criteria for cytological classification of SCC included: 1) Well differentiated- Wide range of maturation in cells with a large number of matured keratinized cells, 2) Moderately differentiated- A large number of round or oval shaped cells with occasional keratinized cells and 3) Poorly differentiated- A large number of immature type of cells with absence of keratinized cells. A modified cytopathological

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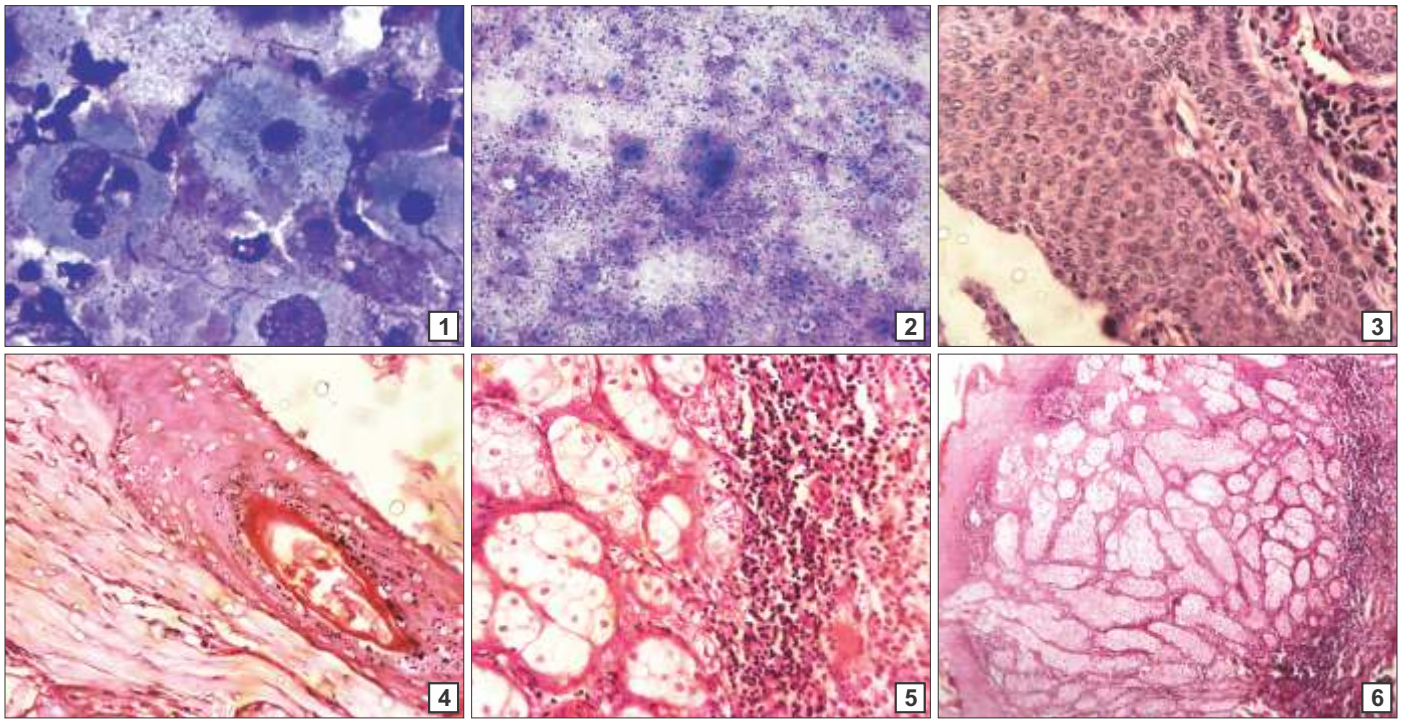

Fig. 1-6. **(1)** Giemsa stained cytosmear showing pleomorphic cells basophilia, multiple nucleoli, vacuolation and increased N:C ratio. Giemsa stain × 1000; **(2)** Giemsa stained cytosmear showing high cellularity, cellular pleomorphism, basophilia, clumping along with increased N:C ratio. Giemsa stain × 100; **(3)** Irregular cords of neoplastic cells, H&E × 100; **(4)** Keratin pearl H&E × 100; **(5)** Infiltration of polymorphonuclear cells, H&E × 400; **(6)** Whorl of neoplastic cells separated by thin fibrous stroma, H&E × 100

criteria given by Alleman and Bain (2000) was followed for cytological features of malignancy.

Representative tissue samples were collected after surgical excision of the neoplastic growth from cases of canine neoplasms. Samples were fixed immediately in 10% neutral buffered formalin and were processed by routine paraffin embedding technique. Sections of 4-5 m thickness were cut using rotary microtome with disposable blade and were stained with routine hematoxylin and eosin (H&E) method (Luna, 1968). The confirmed/suspected cases of nine squamous cell carcinomas were then subjected to Masson's trichrome special staining for keratin and collagen. Criteria for histopathological classification of SCC as per the method described by Chandrasekhar *et al.* (2011) included: i) Well differentiated-Presence of proliferating neoplastic squamous epithelial cells arranged in compact cords or nests of varying sizes, abundant connective tissue and lamellated keratin pearls in the centre of the islands, ii) moderately differentiated- Compactly arranged proliferating cells forming cords or nests of cells separated by thin fibrous stroma with presence of individual keratinized cells and iii) poorly differentiated- Highly proliferating cells showing high anaplasia with absence of cell nests and keratin pearls or keratinized cells. The connective tissue proliferation and keratin formation in SCC were graded as: Absent, +: Minimal, ++: Moderate and +++: Marked.

Cytological examination reveals presence of large numbers of malignant squamous tumour cells are arranged individually or in clumps. The cells are pleomorphic in appearance, shape varies from round to caudate with distinct anisokaryosis and anisocytosis. Anisokaryosis is reflected by variation of nucleus from pyknotic to large type, increase N:C ration, presence of multiple nucleus and nucleolus followed by perinuclear vacuolation (Figs. 1 and 2). The observations were harmonized with the preceding observations (Henson, 2001). The SCC are in different stages of maturation characterised by asynchronous developmental stage of nucleus and cytoplasm, variation in the number of keratinized squamous cells because of varying degree of differentiation were seen during examination. In Giemsa, the cytoplasm of keratinized cells appeared bluish to purplish. The findings were in support with that of previous results (Raskin *et al.*, 2001). There was also presence of polymorphonuclear and mononuclear cells in large numbers (Fig. 1). This can be corelated with the macroscopic observations of lesions either with ulcers or inflammation of haemorrhagic otherwise suppurative type (Andreasen *et al.*, 2001; Raskin, 2001).

The five well differentiated SCC reported in the present study revealed neoplastic cells arranged in the form of irregular cords within the tumour (Fig. 3). Nest of neoplastic squamous cells were surrounded by connective tissue stroma where the keratin producing stratum

corneum layer was present at the centre and produced the typical "epithelial pearl" or "keratin pearl" (Fig. 4). Intensely eosinophilic rings of keratin were present at the centre of the "epithelial pearl". In addition, focal to diffuse areas of necrosis associated with infiltration of inflammatory cells like neutrophils, lymphocytes and plasma cells were also evident (Fig. 5). These findings were in accordance with previous observations (Viswanath *et al.*, 1998; Raskin, 2001). The one moderately differentiated SCC encountered in the present study consisted of proliferating neoplastic cells which were arranged in the pattern of cord or whorland were separated by thin fibrous stroma (Fig. 6). Occasional cell nests were also seen in the deeper tissue with minimal keratinization Moderate infiltration of inflammatory cells was also evident (Viswanath *et al.*, 1998). The three poorly differentiated SCC were characterized by indistinct epithelial layering, indistinct or absent keratinization and whorl or cord formation. Infiltration of inflammatory cells and fibroplasia were also minimal. These findings were in agreement with earlier observations (Viswanath *et al.*, 1998; Lascelles *et al.*, 2000; Raskin, 2001).

The prominent "keratin pearl" formation in well differentiated stage; cord or whorl formation by neoplastic cells associated with minimal keratinization in moderately differentiated stage; and absence of whorl or cord formation along with indistinct or absence of keratinization in poorly differentiated stage are the most effective histopathological parameters. Furthermore, in this study of SCC, the cytological features at different stages would also facilitate the diagnosis of SCC at different stages.

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