

**Original Full Paper****Canine Squamous Cell Carcinoma: a Review of 17 Cases**

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**Abstract**

Cancer, a fatal malignant disturbance of growth is one of the major causes of mortality in canines. Of the many neoplasms that are known to affect dogs, squamous cell carcinoma (SCC) is relatively more common and highly malignant. In the present investigation, 138 cases of tumor or tumor like growths were evaluated and 17 squamous cell carcinomas (SCC) encountered were studied in relation to its occurrence in population, age, sex, breed, gross appearance, location of the lesion, cytological findings, histological observations and immunoreactivity to cytokeratins. Cytological smears revealed large number of malignant squamous cells occurring either individually or in clusters exhibiting pleomorphism, anisokaryosis and anisocytosis. Well differentiated forms, histologically showed cords or nests of proliferating neoplastic cells consisting of immature polyhedral cells at the periphery and eosinophilic lamellated keratin pearls at the centre. The moderately differentiated ones were characterized by proliferating cells forming cords or nests of cells separated by thin fibrous stroma. The varying intensity of immunostaining observed to 34βE12 raised against high molecular weight cytokeratins (1, 5, 10 and 14) correlated well with cellular differentiation with high expression in well differentiated and less in poorly differentiated SCC.

**Key Words:** squamous cell carcinoma, cytology, differentiation, immunoreactivity, high molecular weight cytokeratins

**Introduction**

Neoplasms in dogs are twice more frequent in comparison to man (14), which progress more rapidly and bear similar anatomical and physiological properties, proving them as an excellent animal model for understanding human cancers. Diagnosis of cancer, which is a prime requirement to take up treatment, is achieved in the field of oncology mainly by morphological and microscopic examinations including cytology and immunohistochemical techniques. Originating from squamous epithelial cells, SCC presents varying features from incomplete carcinoma in intra epidermal form to highly malignant tumor type in its invasive form, exhibiting different degrees of differentiation in member cells of its progeny (7, 29).

Grossly SCC occurs as nodular or erosive lesion showing red firm plaque to a cauliflower like ulcerated mass and may be seen in any organ of the body lined by epithelium like skin, eye, oral and nasal cavities, tongue, esophagus, lung, penis, vagina and footpad (4, 5, 6,8, 10, 22, 25, 33, 36, 38). Cytologically, SCC exhibits striking features like anisokaryosis, anisocytosis, perinuclear halo, binucleation, multinucleation and characteristic coloration of cytoplasm with different stains (12, 16, 17). Though cytological as well as histological diagnosis of well differentiated SCC is, in general, an easy task, confirmation of poorly differentiated SCC requires further evaluation employing immunohistochemistry (IHC) to detect specific tumor marker (24).

The advent of diagnostic immunohistochemistry has made a great impact on oncology and has been variously referred to as 'brown revolution' and 'magic markers'. Although, introduced 63 years ago (9), the technique was not readily embraced by diagnostic laboratories until the last two decades. Immunohistochemical detection of tumor markers like cytokeratins and keratins (epithelial cells), vimentin (mesenchymal cells) and desmin (muscle cells) is undertaken to confirm the diagnosis of neoplasms in human and veterinary medicine. Of these many intermediate sized filaments, the cytokeratins that constitute part of the cytoskeleton are expressed by various epithelia and neoplasms arising from them (13, 26, 30, 39). The HMW cytokeratins consisting of CK-1, 5, 10 and 14 are specific markers for squamous cells and their expression in cells is directly correlated with cellular differentiation. The current work was undertaken with the objectives of 1) present the epidemiological characteristics of 17 cases of SCC in dogs, 2) evaluate cytology as a tool for diagnosing SCC 3) characterize SCC based on histopathology. 3) demonstrate the expression of cytokeratins (1, 5, 10 and 14) in canine SCC by immunohistochemistry.

## Materials and Methods

### *Collection of samples:*

A survey of neoplasms in dogs collected from August 2004 to December 2006 was performed at the Department of Pathology, Veterinary College, Hebbal, Bangalore, India. A total of 138 dogs presented to different nearby veterinary hospitals with a history of neoplasm or growths at various locations formed the source for the present study. Particulars like breed, age, sex, color and clinical manifestations exhibited by the animals were recorded.

### *Preparation of cells for cytological examination:*

Tissue samples for cytological examination were collected by various methods from the growths depending upon the type and location of the neoplasms. For nodular superficial growths, fine needle aspiration cytology (FNAC) and fine needle aspiration biopsy (FNAB) were used. For ulcerated and surgically excised neoplastic growths scraping smears were prepared. The smears were stained using standard Giemsa, Toluidine blue, Wright's, Wright-Giemsa and Papanicolaou standard staining techniques (20). 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. Criteria for cytological features of malignancy were adopted (1, 35)

### *Preparation for histopathological examination:*

Tissue samples collected either by biopsy after surgery or at postmortem from cases of canine neoplasms were fixed immediately in 10% neutral buffered formalin. Representative samples from the neoplastic growths were processed by routine paraffin embedding technique. Sections of 4-5  $\mu$ m thick were cut using Bright rotary microtome with disposable blades. These sections were then stained with routine hematoxylin and eosin (H&E) method. The confirmed/suspected cases of 17 squamous cell carcinomas were then subjected to Van Gieson's and Masson's trichrome special staining to show up the amount of keratin material and collagen deposition. Criteria for histopathological classification of SCC included: 1) 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 2) moderately differentiated- Compactly arranged proliferating cells forming cords or nests of cells separated by thin fibrous stroma with presence of individual keratinized cells and 3) 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.

### *Immunohistochemical staining for Cytokeratins:*

The indirect Immuno Peroxidase Test (IPT) was employed for immunohistochemical staining of HMW cytokeratins. Mouse antihuman cytokeratin, HMW, clone 34  $\beta$ E12 shown to react with the 66, 57,51 and 49 KD proteins corresponding to Cytokeratins 1,5,10 and 14 ready-to-use, LSABTM2/ envision TM (Dil Ab 7 ml), ( Cat No. N155330 Dako Cytomation, USA) served as primary antibody. Anti mouse IgG raised in goat conjugated with Horseradish Peroxidase (Dako Cytomation, USA) at a dilution of 1:100 was used as secondary antibody. Diamine Benezdrine tetra hydrochloride and Harris Hematoxylene were used as substrate and nuclear counter stain respectively. Criteria for the evaluation of immunoperoxidase reaction included -: No reactivity (immunoreactivity was absent in all cells), +: Weak reactivity (few cells showing immunostaining or weak coloration in the cytoplasm), ++: Moderate reactivity (most of the cells of cell nests showing immunostaining with areas of both intense and weak colorations) and +++: Marked reactivity (all the cells of the cell islands showing intense brown coloration)

## Results and Discussion

A total of 138 cases suspected for neoplastic growths were screened by cytological and histopathological procedures to diagnose SCC. SCC thus confirmed and those which required further

confirmation were subjected to immunohistochemical evaluation by using monoclonal antibodies known to react specifically with HMW cytokeratin. The results of current study confirmed 17 cases of SCC of varying differentiation. The results of the present study are discussed under the subheadings of epidemiology, macroscopic appearance, cytology, histopathology and immunohistochemistry of SCC.

#### *Epidemiology:*

In the present investigation, it was observed that SCC occurred in all age groups ranging from 2 to 14 years with an average of  $7.73 \pm 0.78$  years and the maximum incidence of 41.18 per cent of SCC was observed in six to nine year aged group of dogs. Among 17 cases, eight were recorded in male and nine in female dogs. Maximum incidence of SCC occurred in nondescript dogs (8) followed by Doberman (2) and German shepherd, Dalmatian, Spitz, Golden Retriever, Cocker Spaniel, Labrador and Poodle (one each). In the present study the most frequently affected site for SCC was gum region and mammary gland with three cases each. The details are summarized in Table 1.

Perusal of the available literature revealed that skin, nasal planum, oral cavity, tongue and digits were the common sites where SCC was reported (4, 5, 22, 36, 38). SCC involving tongue and digits have been studied (6, 8, 10, 25, 33). Incidence of SCC in sites like right humerus (2), anal sac (11), middle ear (43), mammary glands (32), penis (37) and eye (40) has also been documented.

#### *Macroscopic appearance:*

Grossly growths of SCC varied in their size, appeared as round, ovoid, irregular or cauliflower like masses with superficial necrosis and ulcerations. The tumor masses revealed rough surface and pink to light brown color. The observations were in conformity with reported findings (4, 5, 15, 18, 34, 36)

#### *Cytology of SCC:*

Smears revealed 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 characterised by nuclei, varying from pyknotic to large type, variable nuclear to cytoplasmic ratio, binucleation and multinucleation and perinuclear vacuolation were observed (Figure 1) Such observations were in line with the previous observations (12, 16, 17) Further, asynchronous cytoplasmic and nuclear maturation, varying number of keratinized squamous cells depending upon the degree of differentiation of SCC were also observed. The cytoplasm of keratinized cells appeared bluish green in Toluidine blue, bluish to purplish in Giemsa and yellowish orange in Papanicolaou stained smears. These results were well supported with the previous findings (7, 28). Cytological smears also revealed a large number of polymorphonuclear and mononuclear cells. This observation is also well supported (3, 27) and was obvious as most of these tumor masses were

either ulcerative or hemorrhagic or inflamed with suppuration in the present study.

Well differentiated SCC: Well differentiated SCC in the present study presented large number of cells with angulate borders containing homogenous cytoplasm with centrally placed nuclei. The neoplastic cells showed a wide range of maturation ranging from small immature type to highly keratinized cells with the number of latter being predominant. The fully keratinized cells were anucleated with cytoplasm staining basophilic in different stains. The predominant number of matured keratinized and anucleated squamous cells cytologically indicated the higher degree of differentiation of the tumor, a factor also shown by earlier authors (7, 29). The extracellular amorphous substance noted in this study could be the scattered keratin debris as previously indicated (7). The perinuclear halo or vacuolation observed in the present study was attributed to the presence of colorless keratohyaline granules. Similar observation has been reported (29).

Moderately differentiated SCC: Moderately differentiated SCC revealed cytologically larger number of round or oval cells, a few angular cells and occasional keratinized cells with retained nucleus occurring individually, in sheets or in clusters. The presence of occasional keratinized cells and more of immature cells in the smear pointed out the less differentiation of the proliferating cells (29). The other features observed were prominent anisocytosis, anisokaryosis, perinuclear halo, binucleation and multinucleation and increased nuclear to cytoplasmic ratio.

Poorly differentiated SCC: Cytologically the spindle cell type of poorly differentiated SCC showed moderate number of round, oval or spindle shaped cells (Figure 2) with cytoplasmic extensions on either side. The absence of keratinized cells in the smear indicated the poor differentiation of the tumor. However, the diagnosis of poorly differentiated SCC based only on cytological examination is difficult and incorrect and requires confirmation by histopathological and immunohistochemical examinations. The other undifferentiated SCC cytologically revealed round or oval shaped cells with high degree of anisokaryosis, anisocytosis and increased basophilia of cytoplasm which are characteristic of less differentiated tumors (29).

Table 1. Epidemiological and diagnostic features of SCC in canines

Sl. No	Breed	Age (year)	Sex	Region / site	Cytology	Histopathology	Connective tissue deposition	Keratin accumulation	Immuno reactivity
1	ND	7	Female	Back	WD	WD	+++	+++	+++
2	CS	12	Female	Perineal	MD	MD	++	++	++
3	ND	9	Female	Inguinal	MD	MD	++	++	++
4	GSD	10	Male	Gum	WD	WD	+++	+++	+++
5	Labrador	8	Female	Gum	WD	WD	+++	+++	+++
6	ND	6	Male	Ventral neck	WD	WD	++	+++	+++
7	GR	6	Male	Abdomen skin	WD	WD	++	+++	+++
8	ND	4	Male	Ethmoid	PD	SpCC	+	-	+
9	ND	7	Male	Perineal	WD	WD+AC	+++	+	+
10	Doberman	2	Male	Gum (invaded to mandible)	WD	WD	+++	++	+++
11	ND	6	Female	Back	WD	WD	++	++	+++
12	Poodle	11	Male	Lips and nostrils	MD	MD	+++	+	+
13	Dalmatian	2½	Female	Lower abdomen	WD	WD	++	++	+++
14	ND	8	Female	Mammary gland	WD+AC	WD+AC	++	+++	+++
15	ND	14	Female	Mammary gland	WD+AC	WD+AC	+++	++	++
16	Spitz	10	Female	Mammary gland	WD+AC	WD+AC	++	+++	++
17	Doberman	9	Male	Infra-orbital (left)	PD	NKSCC	+	-	+

ND = nondescript, CS = cocker spaniel; GSD = German Shepherd, GR = Golden Retriever, WD = well differentiated, MD = moderately differentiated, PD = poorly differentiated, AC = adenocarcinoma, SpCC = spindle cell carcinoma, NKSCC = nonkeratinizing squamous cell carcinoma

+ = mild  
 ++ = moderate  
 +++ = marked  
 - = absent



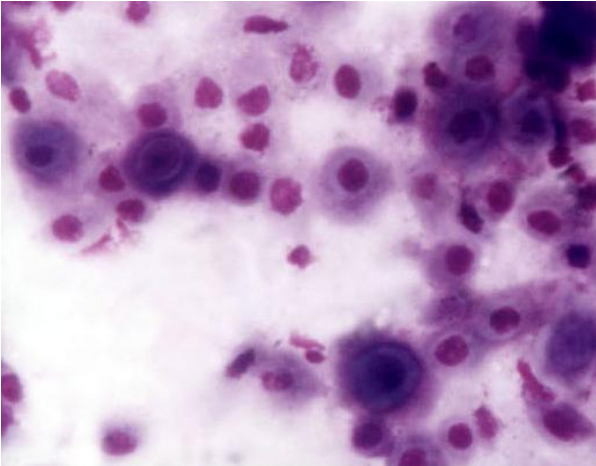


Figure 1: Cytological Smear of a well differentiated SCC showing prominent perinuclear halo in majority of the neoplastic squamous cells GIEMSA, Obj. 50X

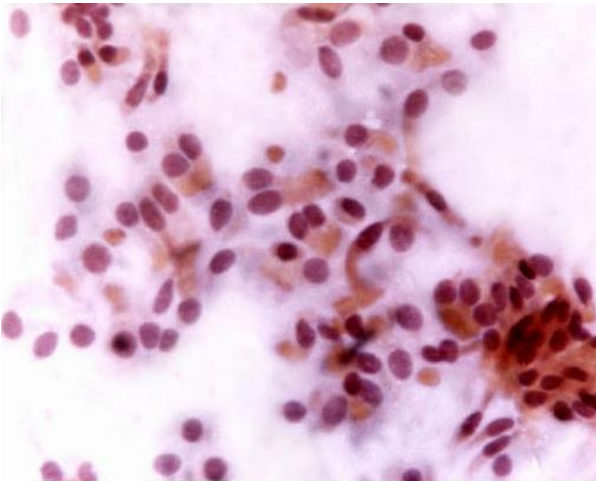


Figure 2: Cytological smear of poorly differentiated SCC of ethmoid region showing many spindle shaped cells PAPANICOLAOU, Obj. 50X

#### *Histopathology of SCC:*

**Well differentiated SCC:** The 12 well differentiated SCC encountered in the present study were featured by cords or nests of proliferating neoplastic cells consisting of immature polyhedral cells at the periphery and eosinophilic lamellated keratin pearls at the centre. The amount of keratin was abundant in all well differentiated types. The proliferating cells revealed moderate cellular pleomorphism, large vesicular nuclei, prominent nucleoli, variable mitotic activity and prominent intercellular bridges (Figure 3). Similar microscopic observations have been reported (11, 43, 19, 21, 32, 36, 41, 42). The lamellated keratin in Masson's trichrome appeared brick red (Figure 4) and yellowish to yellowish green in VanGeison stains. These special stains also facilitated in demarcating the amount of connective tissue that varied from moderate to marked. The connective tissue was particularly abundant around invading cords in deeper areas of the neoplasm. These findings find the support by previous

workers (5, 25) who observed fibroplasia or desmaplasia to be abundant around penetrating epithelial papillae in the deeper areas of the neoplasm. Further the focal to multifocal areas of necrosis and inflammatory changes with lymphocytes, plasma cells and neutrophils were in tune with previous observations (5, 32, 36, 38). In addition, remarked that such changes were found to be anticipated features in ulcerated and fast growing tumors (36).

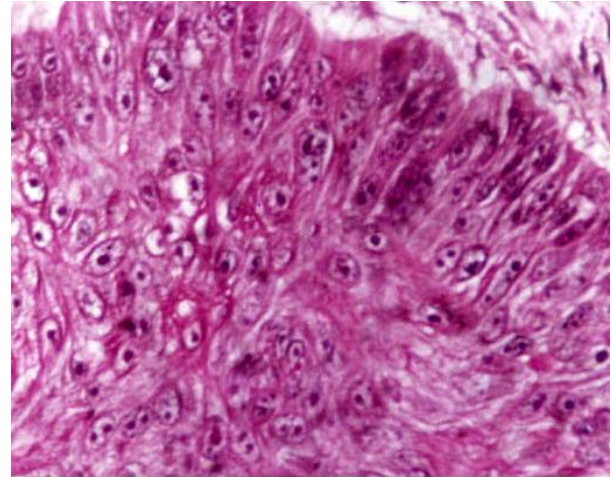


Figure 3: Histopathological section of well differentiated SCC showing prominent intercellular bridges H&E, Obj. 50X

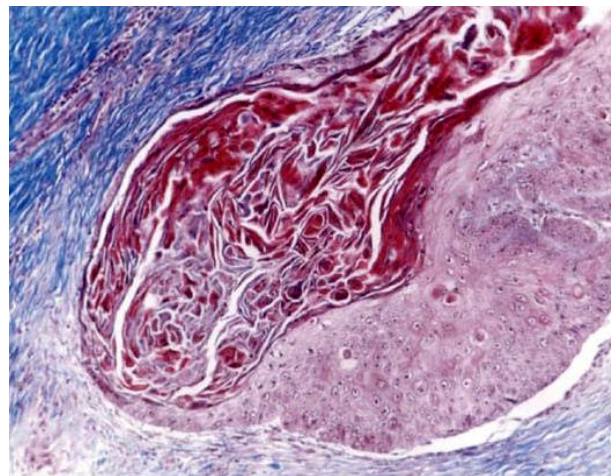


Figure 4: Histopathological section of well differentiated SCC showing brick red colored keratin and intensely blue colored proliferating connective tissue MASSON'S TRICHROME, Obj. 12.5X

In the present study, one case of SCC which showed the presence of cholesterol crystals along with proliferating tissue could be due to excessive abdominal subcutaneous adipose tissue with no direct relationship with the neoplastic mass. The four co-existing SCC and adenocarcinoma of mammary gland and hepatoid gland were of well differentiated type with proliferating glandular tissue and neoplastic squamous cells both occurring together and often separated by thick connective tissue stroma. A metastasized SCC in lung, liver and pancreas gave similar picture as that of primary tumor.

**Moderately differentiated SCC:** The three moderately differentiated SCC were characterized by proliferating cells forming cords or nests of cells separated by thin fibrous stroma, irregularity in cell orientation, loss of prickle cell appearance, prominent mitotic activity, individual cell keratinization vacuolar degeneration in large cells and occasional nests of neoplastic cells invading the deeper tissue which consisted of abundant fibrous stroma. In addition infiltration of lymphocytes, plasma cells and neutrophils was also observed. These findings were in accordance with reported observations (5, 19, 38).

**Poorly differentiated SCC:** Of the two poorly differentiated SCC encountered in the present study one was histologically confirmed as spindle cell type, which comprised highly proliferating large pleomorphic spindle and polygonal cells with abundant pale to eosinophilic vacuolated cytoplasm. Further, it also revealed absence of lobulation or cord formation, occasional whorled arrangement of cells, moderate to high degree of cellular anaplasia, pleomorphism and mitotic activity, minimum fibroplasia and infiltration of inflammatory cells. These findings were in accordance with previous observations (27, 34, 38). In another case, the poorly differentiated SCC appeared as island of moderately pleomorphic cells formed into clusters with no keratinization or cord formation.

#### *Immunohistochemistry of SCC:*

The 17 cases of SCC diagnosed in the present investigation based on cytological and histopathological studies were subjected to immunohistochemistry using anticytokeratin antibodies clone 34 $\beta$ E12 raised against HMW cytokeratins (1, 5, 10 and 14). The varying intensity of immunostaining was correlated with differentiation of cells and extent of expression of these cytoskeletal elements was used in an attempt to grade these SCC. In case of well differentiated SCC, the parabasal and keratinized cells which occupied the centre of the islands of proliferating cells, revealed marked (+++) immunostaining (Figure 5). This could obviously be due to the fact that these cells at the centre were highly matured cells with more cytoskeleton formation. The cells in periphery which were of immature nonkeratinized and highly proliferating type revealed sparse immunoreactivity (+) indicating less formation of cytoskeletal substances in their cytoplasm. This clearly indicated that the cytokeratin formation is directly related to the degree of differentiation of the proliferating cells. The coexisting well differentiated SCC with adenocarcinoma of mammary gland and hepatoid gland revealed similar immunoreactivity with that of independently occurring well differentiated SCC. However, glandular portion of these tumor masses showed no reactivity to anti HMW cytokeratin antibodies used in the study (Figure 6). This observation draws considerable support from previous work (23, 30), who stated that the cells of SCC contain HMW cytokeratins whereas low molecular weight

cytokeratins constitute the cytoskeleton of cells of adenocarcinoma.

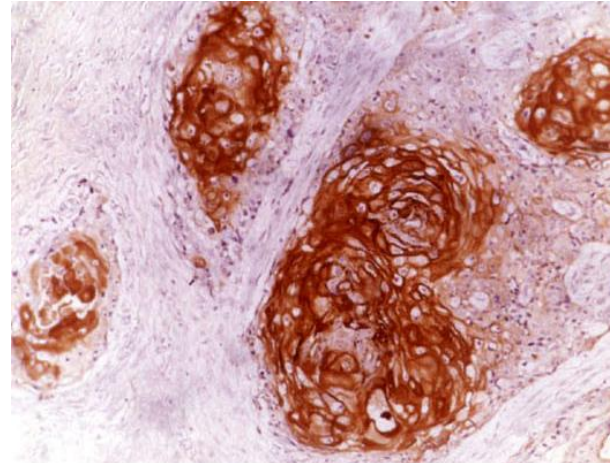


Figure 5: Histopathological section of well differentiated SCC showing intense immunoreactivity in the cell nests and absence of the same in connective tissue stroma IHC, Obj. 12.5X

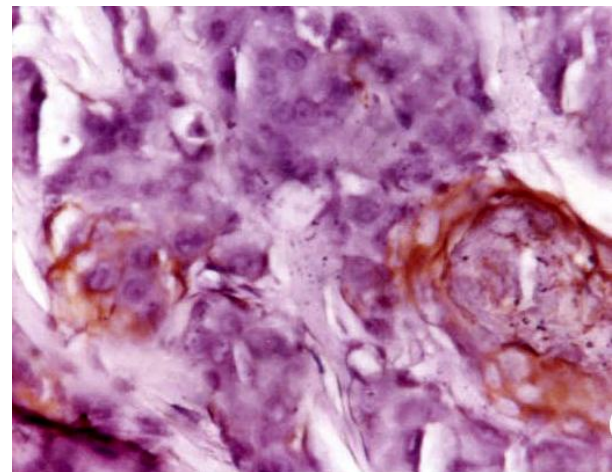


Figure 6: Coexisting SCC and adenocarcinoma of hepatoid gland showing moderate immunostaining only in the SCC component of the neoplasm IHC, Obj. 50X

Moderately differentiated SCC presented similar immunoreactivity like that of well differentiated SCC consisting areas of strong and weak immunoreactivity (Figure 7). From this observation, it could be inferred that the differentiation of SCC based on immunoreactivity as well differentiated and moderately differentiated is difficult as both the types comprise of proliferating cells at different levels of differentiation or maturity and exhibit almost similar type of immunostaining.

The two poorly differentiated SCC which were diagnosed histopathologically, when subjected to immunostaining gave positive reaction only in a few cells lining the innermost layer of cell islands with absence of any immunoreactivity in rest of the proliferating cells. This clearly indicated that the tumor was fast growing and highly undifferentiated with minimum formation of cytokeratin restricted to only those cells which were partially differentiated (Figure



8). It could be observed in the present study that immunohistochemical techniques proved to be useful tools in knowing the cell lineage when neoplasms were poorly differentiated. The expression of cytokeratin was apparently conserved even after transformation to epithelial cells as previously indicated (13, 23, 26, 30, 39). It could be inferred from the present study and previous observations (31, 24) that HMW cytokeratins serve as a useful tool for possible grading of the SCC and knowing the squamous origin of SCC that present as poorly differentiated ones.

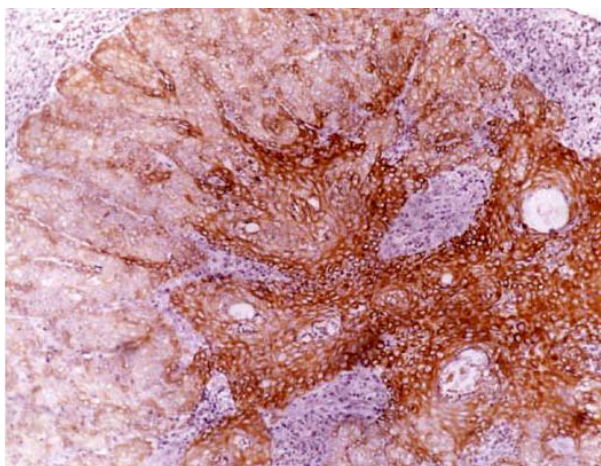


Figure 7: Histopathological section of moderately differentiated SCC showing areas of intensely and moderately stained cells IHC, Obj. 50X

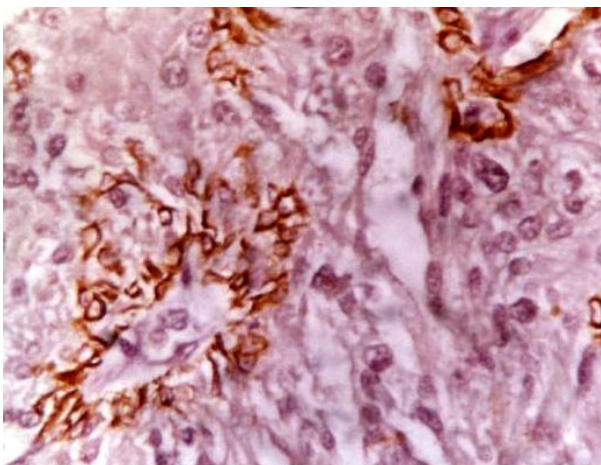


Figure 8: Histopathological section of spindle cell carcinoma showing intense immunoreactivity only in inner layer of cells IHC, Obj. 50X

## Conclusion

Squamous cell carcinoma is a relatively more common and fatal neoplasm in dogs with rapid metastatic spread. In the current investigation, it occurred in different age groups ranging from 2 to 14 years with gum and mammary gland as the frequently affected sites. Grossly, they varied in their size, appeared as round, ovoid, irregular or cauliflower like masses exhibiting superficial necrosis and ulcerations. The cytological and histopathological evaluation

accompanied by immunohistochemical demonstration of extent of expression of HMW cytokeratins (34 $\beta$ E12) not only favored rapid diagnosis and identification of cell lineage but also the degree of cellular differentiation which is of prognostic value to clinicians.

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