RESEARCH ARTICLE

Incidence, Gross Morphology, Histopathology and Immunohistochemistry of Canine Mammary Tumors

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Abstract

The aim of present investigation was to study the gross morphology and incidence of canine mammary tumors (CMTs) based on age, sex, breed, reproductive status and location along with histopathological classification and immunohistochemical characteristics. A total of 56 CMTs samples were collected from 49 cases of dogs. Gross morphology was studied in 26 cases of canine mammary tumors. For histopathological classification, samples were fixed in 10% formalin, embedded in paraffin and sections (5 µm thick) obtained from each was stained with H&E stain. Immuno-histochemistry was carried out by using p63 antibody to confirm the histopathological types of CMTs. Malignant tumors and benign tumors were mostly observed in older dogs. Among 9 breeds affected, the highest incidence was recorded in a German shepherd. Caudal abdominal pair was most commonly affected. Most of the cases were observed in intact female dogs, except for one male dog. The tumors were oval and round in shape with 30–2000 g weight, soft to hard in consistency and grayish white cut surface. Out of 56 CMTs, the highest incidence was found of malignant neoplasms (36/56, 64.28%), followed by benign neoplasms (10/56, 17.85%) and non-neoplastic proliferation hyperplasia/dysplasia (10/56, 17.85%). Complex carcinoma, carcinoma, and malignant myoepithelioma and malignant myoepithelioma were confirmed by p63 antibody. In these neoplasms, myoepithelial cells showed strong immunoreactivity with p63.

Keywords: Canine mammary tumor, Incidence, Gross morphology, Histopathology, Immunohistochemistry.

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Introduction

The mammary gland is a modified apocrine sweat gland found only in mammals and characterized by a tubuloalveolar structure. In dog mostly five pairs are present; two thoracics (M1 and M2), two abdominal (M3 and M4) and one inguinal (M5) pair. Mammary gland tumors are one of the most common neoplasms in female dogs and mostly occur in countries where dogs are not routinely spayed at an early age. Canine mammary tumors are heterogeneous in their pathological feature and clinical behavior and arise from the different type of tissues in the mammary gland (epithelial or glandular tissues, and mesenchymal or connective tissues) (Misdrop *et al.,* 1999). Malignant type of canine mammary tumors is more frequent than benign neoplasms (Shekhar *et al.,* 2001). WHO published the first "International Histological Classification of Tumours of Domestic Animals" in 1974 and its modification was made in 1999. Goldschmidt *et al.* (2011) gave a new histopathological classification of CMTs based on the modification of WHO criteria.

Immunohistochemistry is widely used in the diagnosis of cancers by the detection of tumor-specific antigens expression (Duraiyan *et al*., 2012). During mammary tumorigenesis, cell-specific differentiation markers are usually retained (Pena *et al*., 2014). In the normal canine mammary glands, luminal epithelial cells are characterized by expression of luminal cytokeratins like CK8, CK18, CK19, and CK7 and basal/ myoepithelial cells characterized by expression of cytokeratins like CK5, CK6, CK14, and CK17 in addition to other markers such as smooth muscle actin (SAM), calponin, vimentin and p63 (Gama *et al*., 2003). The p63 has been proposed to be an important myoepithelial cell marker and may indicate myoepithelial histogenesis in some tumors of the canine mammary gland (Gama *et al*., 2003). This study was aimed to evaluate the gross morphology and incidence of canine mammary tumors along with histopathological classification and immunohistochemical characteristics.

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Materials and Methods

A total of 56 canine mammary tumors (CMTs) samples collected from 49 (multiple lobes affected) dogs presented at Veterinary Clinical Complex (VCC) and Department of Veterinary Surgery and Radiology of Veterinary College,

Anand, as well as government and private veterinary clinics of Gujarat state, formed the study material.

Clinical History Gross Morphology, and Histopathological Classification

The primary report for the case was reviewed for sex, age, breed, the weight of the dog, reproductive status (intact or spayed), the location of the tumor and also a number of mammary glands affected. Preoperatively, gross examinations of the CMTs were carried out. Tumor masses were examined for its mobility (mobile or fix), ulceration or discharge and also for a location with respect to the longitudinal axis (thoracic, abdominal or inguinal mammary gland). After surgical excision, shape (round, oval, irregular, multilobulated, etc.), weight (g), consistency (soft, hard or firm, cystic, etc.) and color of a cut surface of the tumor were examined. All the tissue samples were fixed in 10% neutral buffered formalin and processed for dehydration, paraffin embedding, section cutting (5 µm) and staining with hematoxylin and eosin. The stained sections upon examination were classified according to the diagnostic criteria proposed by Goldschmidt *et al*., (2011) for mammary tumors of dog.

Immunohistochemistry

For immunohistochemistry 4.0–4.5 µ sections of formalin fixed paraffin embedded specimens were cut and taken on Poly-L-Lysine coated slides and stained as per the routine method of immunohistochemistry (Im *et al.,* 2014). In the present study, p63 antibody (Clone/Isotype–DAK-p63/IgG2a, kappa, Redy to use, Dako, Denmark) was used. Antigen retrieval was performed by citrate buffer, pH 6 by using a pressure cooker. Canine mammary tumor samples, known to express markers, were used as positive controls for p63. Nuclear staining of more than 5% of myoepithelial cells for p63 was considered positive.

Results and discussion

Incidence and Clinical Findings

The mean (±SD) age of all affected dogs was 8.60±2.59 years (range 2.5–15 years) at the time of surgery. The mean age of dogs with ectasia/hyperplasia/dysplasia was 5.8±1.72 years (range 2.5–8 years), and those with benign tumors and malignant tumors was 9 ± 1.19 years (range 7–11 years) and 9.08 ± 2.64 years (range 4–15 years), respectively. In an earlier study also the non-neoplastic proliferation was found in young age female dogs (4.4 years), while incidences of benign (7.3 years) and malignant tumors (8.2 years) were increased with advanced age (Raval *et al.,* 2018). In another study, mean age of dogs with hyperplasia/dysplasia, benign tumors, and malignant tumors was 9.0 ± 2.7 years (3–16 years), 8.6 ± 2.6 years (1.6–16 years), and 9.6 ± 2.6 years (3–9 years), respectively (Im *et al.,* 2014).

Among the 9 breeds of dogs presented, most cases occurred in German shepherd (15), followed by Pomeranian (10), Labrador retriever (8) and non-descript (6). Other affected breeds were Great Dane (4), Neapolitan Mastiff (1), Dachshund (3), Cocker spaniel (1) and Alsatian × Labrador retrieval (1). All the affected breeds were pure, except one mix breed. Breed predisposition is based on a different geographical area because different areas have a different pattern of breed distribution. Present observations in Gujarat concurred with previous reports from other parts of India (Reddy *et al.,* 2009; Raval *et al.,* 2018).

Out of 49 cases of canine mammary tumor, 48 cases were of female dogs and one male. Of the 28 cases with known reproductive status (spayed or intact), most dogs were intact, except three. In a previous study out of 40 clinical cases suspected of canine mammary tumors, 38 were diagnosed as CMTs in females and two cases were noticed in male dogs (Lather *et al.,* 2017). Dogs that were overio-hysterectomized before the first estrus had only 0.5% risk of developing mammary tumors, but if dogs were overio-hysterectomized between the first and the second estrus or after the second estrus the risk was 8% and 26%, respectively (Schneider *et al.,* 1969).

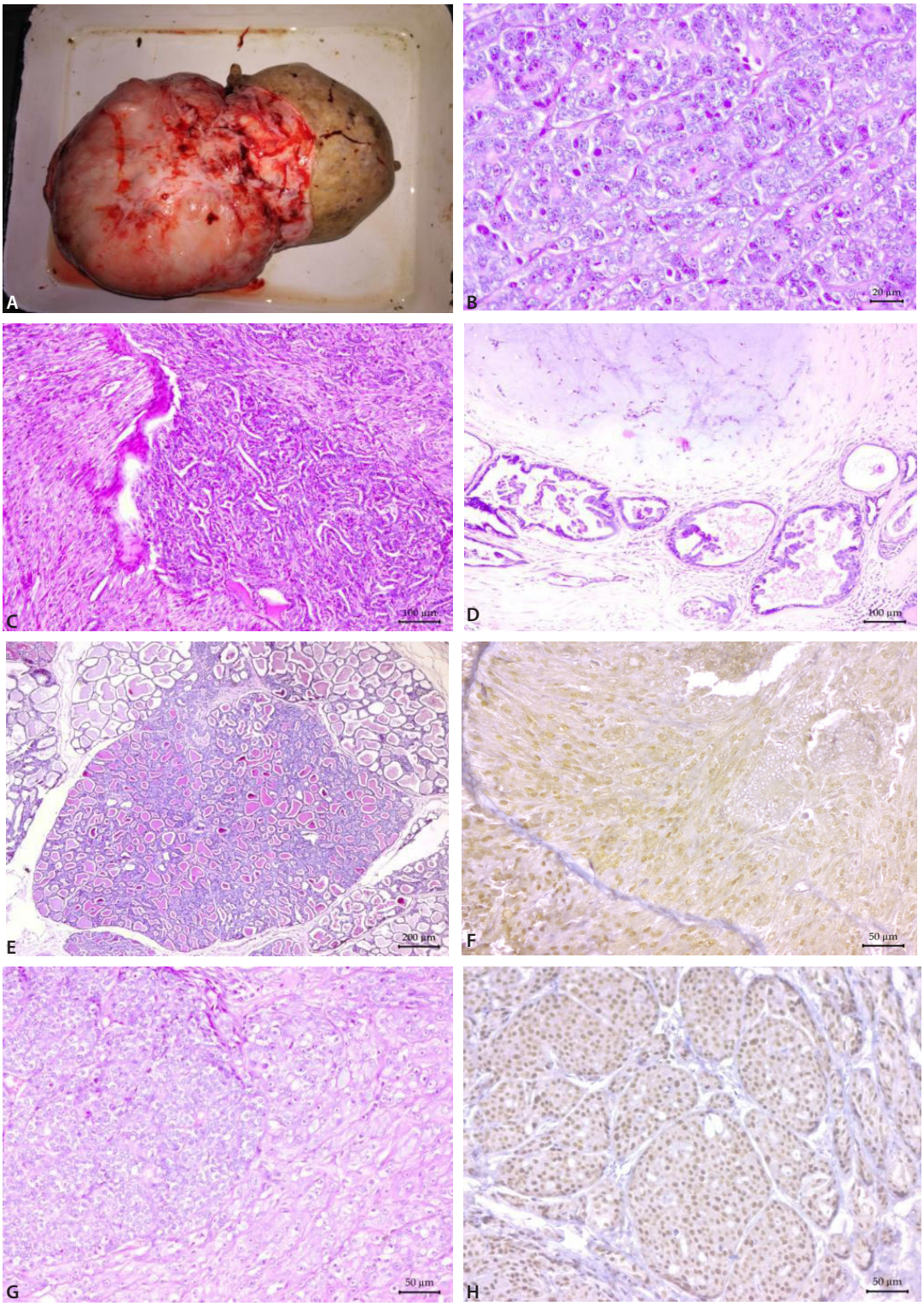
Most of the CMTs (n = 35) were recorded in cranial abdominal, caudal abdominal and the inguinal lobe of the mammary gland. The highest incidence of CMTs was recorded in caudal abdominal (n = 14), followed by inguinal (n = 12), cranial abdominal (n = 11), caudal thoracic (n = 3) and cranial thoracic (n = 3) glands. In the previous study, the highest incidence of the mammary tumor was recorded in inguinal pair followed by caudal and cranial abdominal pair and caudal and cranial thoracic pair (Raval *et al.,* 2018).

Gross Morphology

Gross morphology was studied in 26 cases out of a total of 49 cases of CMTs. The shape of most of the mammary tumors was oval (10) (Fig. 1A), followed by round shape (9), irregular (3), multilobulated (3) and cylindrical (1). The weight of tumor masses ranged from 30 g to 2000 g. Most of the tumors were soft to hard in consistency (9), followed by hard (6), elastic and fluctuate (4), soft (3), soft and elastic (3) and elastic (1). Cut surface of the different tumor was grayish white (9), white (6), reddish white (3), brownish black (2), black (2), and one case each of pinkish, pinkish white, grayish red and grayish tan color. Majority of tumors were fixed, except five which were movable. In six cases ulcers were present on the surface of tumors. All these findings concurred well with the previous study of Acharya *et al.* (2017).

Histopathological Classification

The classification of 56 CMTs is summarized in Table 1. Total 56 tumors from 49 cases of CMTs (multiple lobes of mammary gland affected) were classified into hyperplasia/dysplasia, benign neoplasms and malignant neoplasms based on the criteria given by Goldschmidt *et al*. (2011).



Figs 1A to H: (A) Oval shape spindle cell liposarcoma weighing 2000 g; (B) Carcinoma – tubular, Neoplastic cells have indistinct cell border with round to oval nuclei along with mild anisokaryosis and anisocytosis. H & E X 400; (C) Complex carcinoma showing malignant proliferating epithelial cells arranged in tubules and benign proliferation of myoepithelial cells, supported by fibrovascular stroma. H & E X 100; (D) Benign mix mammary tumour. Focal extensive area of cartilage with proliferated neoplastic epithelial cells form a irregular tubules, myoepithelial cells arranged periphery of the tubules and supported by fibrous stroma. H & E X 100; (E) Lobular hyperplasia with secretory activity. Moderate dilation of acini and ducts with variable amount of eosinophilic secretory material. H & E X 50; (F) Complex carcinoma. Myoepithelial cells showing immunoreactivity with p63. Immunoperoxidase staning, DAB chomogen, Gill's hematoxylin counterstain. X 200; (G) Malignant myoepithelioma. Neoplasm composed of oval to spindle shaped malignant myoepithelial cells. H & E X 200: (H) Malignant myoepithelioma. Neoplastic oval shape myoepithelial cells showing strong immunoreactivity with p63. Immunoperoxidase staining, DAB chromogen, Gill's hematoxylin counterstain. X 200.

This classification includes the five new morphological subtypes of Canine mammary carcinomas as compared to the 1999 WHO classification, viz., Micropapillary invasive carcinoma, Comedocarcinoma, Ductal carcinoma, Intraductal papillary carcinoma, Carcinoma, and Malignant myoepithelioma. Similar classification of CMTs was also made by Raval *et al.* (2018) and Im *et al.* (2014). In the present study, out of 56 CMTs, the highest incidence was found of malignant neoplasms (36/56, 64.28%), followed by benign neoplasms (10/56, 17.85%) and non-neoplastic proliferation hyperplasia/ dysplasia (10/56, 17.85%) (Table 1). Similar classification and observations have been reported by previous workers also (Reddy *et al.,* 2009; Chavan *et al.,* 2016).

Table 1: Incidence and histopathological classification of canine mammary tumors

Type of proliferation	*No.*	*%*
(a) Hyperplasia/dysplasia		
Duct ectasia	1	1.78
Lobular hyperplasia-Regular	2	3.57
Lobular hyperplasia with secretory activity	3	5.35
Lobular hyperplasia with fibrosis	4	7.14
Total	10	17.84
(b) Benign neoplasms		
Ductal adenoma	2	3.57
Fibroadenoma	1	1.78
Complex adenoma	2	3.57
Benign mix tumour	3	5.35
Lipoma	2	3.57
Total	10	17.84
(c) Malignant neoplasms		
Carcinoma - tubular	9	16.07
Carcinoma - tubulopapillary	2	3.57
Carcinoma - cystic-papillary	1	1.78
Carcinoma - complex type	3	5.35
Carcinoma and malignant myoepithelioma	4	7.14
Carcinoma- Mixed type	5	8.92
Ductal carcinoma	2	3.57
Intraductal papillary carcinoma	4	7.14
(d) Malignant epithelial neoplasms: Special type		
Malignant myoepithelioma	1	1.78
Adenosquamous carcinoma	1	1.78
(e) Malignant mesenchymal neoplasm: Sarcoma		
Osteosarcoma	2	3.57
Fibrosarcoma	1	1.78
Spindle cell liposarcoma	1	1.78
Total	36	64.23
TOTAL (10+10+36)	56	100

The incidence of malignant neoplasms was more as compared to benign neoplasms and hyperplasia/dysplasia. In malignant neoplasms, commonly observed neoplasms were carcinoma-tubular (Fig. 1B), followed by carcinoma-mixed type, carcinoma, and malignant myoepithelioma, intraductal papillary carcinoma and complex carcinoma (Fig. 1C and Table 1). Carcinoma-mixed type was reported as prominent neoplasm among the malignant tumor of CMTs (Raval *et al*., 2018; Im *et al*., 2014; Reddy *et al*., 2009). In the present study, one case of complex carcinoma was found in the male dog. In the past, more number of benign neoplasms and malignant carcinosarcoma were reported in male dogs (Lather *et al*., 2017; Han *et al*., 2016). In benign neoplasms, benign mix tumors (Fig. 1D) were most commonly observed, which was similar to past observation (Im *et al*., 2014) and lipoma found as benign mesenchymal neoplasm in the present investigation was supported by the previous finding of Raval *et al.* (2018). In hyperplasia/ dysplasia, lobular hyperplasia with fibrosis and lobular hyperplasia with secretory activity (Fig. 1E) were most commonly found (Table 1).

Immunohistochemistry

By IHC results for the p63 antibody, complex carcinoma (Fig. 1F) (3), carcinoma and malignant myoepithelioma (4) and malignant myoepithelioma (Figs 1G and H) (1) were identified out of 32 malignant mammary carcinomas. In these neoplasms, nuclei of mayoepithelial cells showed positive immunoreactivity with p63. Immunohistochemistry plays an important role in the accurate diagnosis of CMT. Calponin, SMA and p63 are used to identify the myoepithelial cells in CMTs. Calponin and SMA also stain the stromal myofibroblast and vascular smooth muscle cells, whereas p63 is a highly specific nuclear myoepithelial marker, and is not expressed by stromal myofibroblast and vascular smooth muscle cells (Pena *et al*., 2014). In past studies also, calponin, p63, SMA were used for the diagnosis of CMT (Raval *et al*., 2018; Bearss *et al*., 2012 and Gama *et al*., 2003).

Conclusion

Malignant and benign tumors were mostly observed in older dogs. Most of the cases of canine mammary tumors were observed in female dogs and caudal abdominal pair was commonly affected. German shepherd was the most commonly affected breed. Most of the tumors were oval and round in shape with 30–2000 g weight, soft to hard in consistency and grayish white cut surface. Majority of tumors were fixed. The incidences of malignant neoplasms were more than benign neoplasms and hyperplasia/dysplasia. In complex carcinoma, carcinoma and malignant myoepithelioma and malignant myoepithelioma, myoepithelial cells showed strong immunoreactivity with p63.

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