Incidence and Histopathological Studies on Tumours of Dog in Bengaluru, India

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ABSTRACT

The current research work was undertaken with the objective of evaluating incidence, and histological study of canine tumours. 68 samples suspected for neoplasia from cutaneous and mammary gland origin were collected from dogs over a period of six months and were classified according to WHO classification (2002). Tumours of mesenchymal origin showed an incidence of 76.47 per cent and incidence of epithelial origin at 23.53 per cent of dogs. The highest incidence of tumours was found in female dogs than in male dogs. Age wise incidence of tumours was found between the range of 2-15 years with a mean age of 8.3 years. The breed wise incidence of tumours was highest in Labrador retriever (22.06%), followed by non-descriptive breeds (19.12%), Golden retriever (17.65%), German Shepherd dog (11.8%), Pomeranian (7.35%), Lhasa Apso (4.41 %), Daschound (4.41%), Boxer (2.94%), Saint Bernard (2.94%), Doberman (2.94%), Mudhol hound (2.94%) and Rajapalyam (1.44%). Based on histopathological classification, the incidence of cutaneous tumours was 77.94 per cent and mammary gland tumours were 22.06 per cent. Among cutaneous tumours, Mast cell tumour (17.64%) occurrence was highest followed by Hemangiosarcoma and Hepatoid gland carcinoma at 8.82% each. The occurrence of mammary gland tumours were also recorded and classified as Tubular (8.82%), Papillary (4.41%), Papillary cystic (5.88%) and carcinosarcoma (2.94%).

Keywords
Canine tumours, Incidence, Histopathology, Epidemiology

Introduction

In recent times, the incidence of cancer in dogs has seen as an upward trend, which might be resulting from increase in their life expectancy and advancements in veterinary diagnostic medicine (Komazawa et al., 2016). Cancer is a disease of the genome, arising from DNA alterations that dysregulate gene structure or function. It is one among the leading cause of death in dogs aged around 10 years old, with 50% of them developing the
disease and approximately one in four succumb to mortality (Adams et al., 2010). Several studies have shown that the cancer is among the common fatal disease, reporting deaths approximately in 15-30% of dogs and in 26% of cats. (Walter et al., 1992) (Eichelberg et al., 1967) (Bonnett et al., 1997).

Knowledge of the distribution of cancers in certain breeds and by age group and sex can provide a basis for veterinarians to consider possible diagnoses. However, the real challenge existing is in determining the number of cancer cases in dogs every year accurately. Recent studies on an overall incidence of cancer in purebred dogs are available in different geographical areas (Brondon et al., 2010), but an incidence rate in cosmopolitan cities in India is lacking. Few studies have reported on an elevated incidence of certain cancers in numerous purebred dogs, such as histiocytic tumours in Bernese mountain dogs, hemangiosarcoma in Golden Retrievers, cutaneous mast cell tumours in Pugs, T-cell lymphoma in Boxers (Dobson, 2013).

In the current scenario, for a fast-growing city like Bengaluru, where the pet population is increasing day by day, there is a need for availing canine registry data for monitoring the status of cancer in dogs. Cancer registry data can be analysed in epidemiological studies using quantitative comparison of tumour types revealing unusual cancer frequencies, providing the directions for research and generation of hypotheses of cancer causation in a specific area which might lead us to identify risk factors (Proschowsky et al., 2003) (Baioni et al., 2017). Thus, this basic information on the occurrence and distribution of the many different neoplastic diseases in dogs is needed and should be made available through a cancer registry (Brondon et al., 2010). This allows diligent veterinarians to monitor individuals from at-risk breeds, leading to earlier diagnoses and more effective treatment (Davis et al., 2004).

Further, in this article, we report cancer incidence rates in domestic dogs within the geographical area of Bengaluru, without any known specific environmental exposure.

**Materials and Methods**

This current study involved collection of tumour specimens which were submitted to private veterinary diagnostic laboratory. A total of 68 surgically resected tumour cases were collected and recorded. Clinical data of cases such as history, species, breed, age, sex, location, size and shape were recorded. The tissue samples were fixed in Neutral buffered formalin for 48 hrs and processed accordingly and stained as per standard procedures with Haematoxylin and Eosin (Bancroft et al., 2008). The tumours were histologically classified under light microscopy according to WHO classification (Goldschmidt et al., 1998) (Goldschmidt et al., 2011).

**Results and Discussion**

**Histogenesis classification**

Out of 68 clinical cases received and examined, the tumours were classified according to their histogenesis viz-a-viz epithelial and mesenchymal origin (Table 1) which constituted 23.53% and 76.47% respectively (Fig.1).

**Benign v/s Malignant neoplasm**

The incidence of tumours in dogs were classified based on their malignancy (Fig.2). The occurrence of malignant tumours was found higher with a total of 84% of the samples received whereas the benign tumours constituted the remaining 16%.
Sex wise classification

The sex wise incidence of tumours is represented in Fig.3. The incidence of cancer in female dogs (61%) was higher than the male dogs (39%).

Age wise classification

The mean age of incidence of cancer is 8.3 years with a range of 2 to 15 years. The incidence of cancer was highest in the 9 to 12 years age group (39%) followed by 6 to 9 years age group. The occurrence is least in youngest, 0 to 3-year age group (Fig.4).

Breed wise classification

The incidence of tumour was found highest in Labrador retriever (22.06%), followed by non-descriptive breeds (19.12%), Golden retriever (17.65%), German Shepherd dog (11.8%), Pomeranian (7.35%), Lhasa Apso (4.41 %), Daschound (4.41%), Boxer (2.94%), Saint Bernard (2.94%), Doberman (2.94%), Mudhol hound (2.94%) and Rajapalyam (1.44%)(Fig.5).

Histopathology incidence wise

The tumour samples were classified based on their histological features as cutaneous and mammary gland tumours in accordance with WHO guidelines (Fig. 6). Among cutaneous tumours, mast cell tumour (n=12, 17.64%, Fig.7a) was ranked first, which showed the presence of neoplastic round cell tumour with well-differentiated cells arranged in rows or small groups, separated by mature collagen fibres of the dermis. Cells were round and monomorphic with ample cytoplasm; most had distinct cytoplasmic boundaries and medium-sized, intracytoplasmic granules and showed eosinophil infiltration. Followed by mast cell tumor was hemangiosarcoma (n=6, 8.82%) which was ranked second. The hemangiosarcoma (Fig.7b) showed for the presence of neoplastic spindle or polygonal to ovoid lining cells, forming poorly recognizable vascular clefts having prominent bulging nuclei. Wherein neoplastic cells of hepatoid gland origin (Fig.7c) showed occasional hepatoid differentiation with cytoplasm that stains less eosinophilic and is vacuolated. The neoplastic cells also showed peripheral invasion involving the underlying skeletal muscles with basaloïd cells showing greater nuclear pleomorphism and hyperchromatism with proliferation suggesting hepatoid gland carcinoma with peripheral invasion. Plasmacytoma (n=5, 7.35%) and Histiocytoma (n=5, 7.35%) was ranked third. Plasmacytoma (Fig.8a) showed for the presence of sheets of round cells with pleomorphic nuclei arranged in a poorly defined cords and nests. These cells showed hyperchromatic nuclei with scant to moderate eosinophilic cytoplasm with some cells showing peri-nuclear clear zones (Golgi) and circular cytoplasmic packets (Russell Bodies) with low mitotic count. Malignant melanoma (n=4, 5.88%), Basal cell carcinoma (n=4, 5.88%), Squamous cell carcinoma (n=4, 5.88%) and Squamous papilloma (n=4, 5.88%) was ranked fourth. Malignant melanoma (Fig.8b) showed the presence of neoplastic cells of melanocytic origin characterized by forming islands of poorly differentiated cells showing remarkable nuclear pleomorphism with moderate to scant eosinophilic cytoplasm. These neoplastic cells showed centrally located prominent nucleolus. Mitotic figures were frequent indicating highly proliferating neoplasia with minimal amounts of melanin pigments within cytoplasm as well as extra-cellular. Basal cell carcinoma (Fig.8c) showed for the presence of well circumscribed intra-dermal neoplastic basal cells with scant cytoplasm. The tumor was moderately multilobulated, with some individual lobules separated by a fibrous
stroma. The individual neoplastic cells were small, round to polyhedral in morphology and arranged in a palisade fashion. The nuclei were ovoid and few mitotic figures were observed. Squamous cell carcinoma (Fig. 9a) showed infiltration of inflammatory cells involving both epidermis and dermis. The Squamous epithelium was disorderly arranged, admixed with huge necrotic debris and haemorrhage involving the surrounding tissues, obscuring most of epithelial cells. Squamous epithelium in certain islands showed nuclear pleomorphism and were large ovoid with vesicular nuclei with central prominent nucleoli and distinct borders. Distinct areas of Keratin “pearls” of laminated keratin were observed indicating a well differentiated neoplasia. Squamous papilloma (Fig. 9b) showed for the presence of circumscribed, unencapsulated, shallow, bowl-shaped endophytic, neoplastic proliferation of the surface epithelium compressing and displacing adnexal structures in the underlying dermis. Cross-sections of the tumor mass showed neoplastic cells arranged in broad infolds and papillary projections supported on thin fibro vascular cores. Leiomyosarcoma, transmissible venereal tumour and cavernous haemangioma (Fig. 10b) were ranked fifth (n=3, 4.41%). Leiomyosarcoma (Fig. 9c) showed the presence of infiltration of inflammatory cells into the lingual structures involving the longitudinally arranged skeletal muscles. The surface epithelium was found eroded with infiltration of neutrophils and lymphocytes. Certain areas showed presence of atypical round to spindle shaped smooth muscle cells with nuclear pleomorphism and scant to moderate cytoplasm, arranged in a disorderly manner. Where in, Transmissible venereal tumour (Fig. 10a) showed for the presence of loosely composed sheets of round cells with relatively round to ovoid shaped and indistinct cell margins. The nucleus was large and round with centrally placed nucleolus surrounded by margined chromatin. The cytoplasm was amphophilic and sometimes shows clear vacuolations. Fibrosarcoma, Myxoma cutaneous, sebaceous gland adenoma and sebaceous gland duct cyst was ranked sixth (n=2, 2.94% each). Fibrosarcoma (Fig. 10c) showed the presence of closely packed, mixed pattern of arrangement of neoplastic cells as densely packed swirling spindle cells alternate with areas populated with few cells and dense conjunctive fibres. Fingerprint whorls of spindle cells were seen too. The neoplastic cells showed pleomorphism and had spindle-shaped to oval or round nuclei with vesicular to hyperchromatic chromatin and eosinophilic to amphiphilic cytoplasm with variable amounts of the collagenous stroma. Mitotic figures were frequent. Myxoma cutaneous (Fig. 11a) showed the presence of an unencapsulated proliferation of stellate to spindle- shaped fibroblasts loosely arranged in an abundant myxoid matrix. The tissue showed low cellularity and mitoses were rare, and there was little or no cytological atypia. Sebaceous gland adenoma (Fig. 11b) showed the presence of neoplastic cells located in multiple lobules separated by connective tissue trabeculae. At the periphery of the lobules, a rim of small, basophilic reserve cells, with hyperchromatic nuclei and scant cytoplasm were observed. The reserve cells were of one cell layer in thickness and were found differentiating into mature sebocytes, with abundant pale eosinophilic, vacuolated cytoplasm and a small central hyperchromatic nucleus. Sebaceous gland duct (Fig. 11c) showed for the presence of an intradermal cyst lined by a thin squamous epithelium (sebaceous duct) and surrounded by hyperplastic sebaceous glands. The tissue was located within dermis with cystic walls composed of basaloid cells and squamous cells with no malignancy. Hemangiopericytoma (n=1, 1.47%, Fig. 12a) was ranked seventh with the presence of
closely packed, mixed pattern of arrangement of neoplastic cells as perivascular whorls and stag horn form by fusiform cells arranged as fingerprint whorls. The neoplastic cells were uniform in appearance with mild to moderate pleomorphism and had spindle-shaped to oval / round nuclei with vesicular to hyperchromatic chromatin and eosinophilic to the amphophilic cytoplasm forming recognizable vascular clefts. The cells lining the clefts have prominent, bulging nuclei which are pleomorphic.

Among mammary gland tumours (Table 3), tubular form of tumour has highest occurrence (8.81%) followed by papillary cystic form (5.88%), papillary form (4.41%) and carcinosarcoma (2.94%).

**Table.1** Classification based on origin of tissue

| Origin of tissue       | No. of cases | In percentage |
|------------------------|--------------|---------------|
| Epithelial origin      | 16           | 23.53%        |
| Mesenchymal origin     | 52           | 76.47%        |

**Table.3** Occurrence of mammary gland tumours in dogs

| Mammary gland tumours      | Occurrence (%) |
|----------------------------|----------------|
| 1) Carcinoma:              |                |
| i) Tubular:                |                |
| • Simplex                  | 8.81           |
| • Complex                  |                |
| ii) Papillary              | 4.41           |
| iii) Papillary cystic      | 5.88           |
| 2) Carcinosarcoma:         | 2.94           |

**Figure.1** The tumour origin wise incidence in dogs, constituted 23.53% of epithelial origin and 76.47% of mesenchymal origin.
**Figure 2** The malignancy wise incidence of tumours in dogs, constituted 16% of benign and 84% of malignant

**Figure 3** The sex wise incidence of tumours in dogs

**Figure 4** The age wise incidence of tumours in dogs
Figure 5: The breed wise incidence of tumours in dogs

Figure 6: Histological classification of tumors

Fig. 7a: Mast cell tumor
Fig. 7b: Hemangiosarcoma
Fig. 7c: Hepatoid gland carcinoma
The current study reported a higher incidence of tumours of mesenchymal origin (76%) and lower incidence of epithelial origin (24%) compared to a Korean study which reported mesenchymal tumours at 38.90 per cent and epithelial tumours at 56.95 per cent (Pakhrin et al., 2007). Similar observations were reported by a Brazilian study which reported occurrence of mesenchymal tumours at 51.92 per cent and epithelial tumours at 42.74 per cent (Machado et al., 2018).

The present study revealed a higher incidence of malignant form of tumours with 84 percent and lower incidence of benign form at 16 percent in comparison with other studies, which reported the incidence of malignant and benign neoplasms in canines at 51.86% to 52.29% followed by 47.70 to 48.14 percent respectively (Simeonov et al., 2011 and Gupta et al., 2009).

Sex wise incidence of tumours in the current study, showed higher predilection towards females (61%) compared to males (39%) which was in accordance with other studies. These studies reported an almost 3-fold higher incidence for all cancers that were observed in female than in male dogs, which could be explained by high incidence of mammary gland tumours observed in female dogs (Merlo et al., 2008). A similar study also reported a comparable higher incidence of tumours in female dogs at 62 percent than in male dogs at 38 per cent (Brondon et al., 2010).

Age wise incidence of tumours in the current study reported a mean age of 8.3 years with a range of 2 to 15 years and the highest tumour incidence was reported in the age range of 9 to 12 years. This age reliant tumour incidence was also reported in other studies as the incidence of development of neoplasm was relatively low in younger animals and increases sharply after the age of 3, peaked at 9 years (Boerkamp et al., 2014). Similar findings were also reported where the mean age of tumour incidence for female dogs was 8.8 years and for male dogs is 7.9 years (Brondon et al., 2010). However, other studies reported that the age of the dogs with the ten most frequent tumours had a mean age of 8.3 years, with a range of 2 months to 19 years (Pakhrin et al., 2007).

Breed wise there was an increase in the incidence of cancer in Labrador Retrievers, which ranked highest among all the dog breeds followed by Non-descriptive breeds, Golden Retrievers, German Shepherd and Pomeranians. The earlier reports from Japan suggested that incidence of neoplastic conditions was found highest in Dachshund, Golden Retrievers and Labrador Retrievers (Komazawa et al., 2016). The current findings of increased incidence of tumours in Golden Retrievers could be due to high population of...
specified breed and popularity among the pet owners in the respective geographical location.

Among the different tumours, incidence of cutaneous tumours ranked first followed by mammary gland tumours which was also reported in earlier studies (Meuton et al., 2003) (Tzvetkov, 1998). Similar observations were also made and reported that skin and soft tissues were by far the most common sites for tumour development and was seven times more common than tumour development in the mammary gland (Dobson et al., 2002).

In the current study, among cutaneous tumours it was observed that the incidence of mast cell tumours (14.7%) was highest followed by Hemangiosarcoma (8.82%), Hepatoid gland carcinoma (8.82%) and Plasmacytoma (7.35%), Malignant melanoma (5.88 %). Similar observations were reported where in the incidence of cutaneous tumors comprised of mastocytoma (14.7%), lipoma (7.48%) and Haemangioma (5.35%). The most frequently diagnosed tumours from the skin epithelial and melanocytic group were hepatoid gland adenoma (9.3 %), squamous cell carcinoma (8.6 %), hepatoid gland carcinomaina (5.34 %) and basalioma (5.11 %) whereas frequently diagnosed canine mesenchymal skin and soft tissue tumours were lipoma (5.58 %), fibrosarcoma (4.41 %), hemangiopericytoma (2.32 %) and haemangiomatous (2.09 %) (Simeonov et al., 2011) (Machado et al., 2016).

Among mammary gland tumours, the tubular form (8.81%) was found highest followed by papillary cystic form (5.88%), papillary form (4.41%) and carcinomascarcoma (2.94%). Similar incidence was reported wherein the tubular form of canine mammary tumor were ranked first (Anjan et al., 2011).

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