RESEARCH ARTICLE

Cytological, Histopathological and Haematological Evaluation of Canine Mammary Tumours: A Comparative Study with Human Breast Cancer

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Abstract

Canine tumours, particularly mammary gland tumours, are among the most common neoplastic conditions in dogs, paralleling the frequency and complexity of human breast cancer. This study was designed to evaluate canine tumours through cytological, histopathological, and haematological techniques, while also drawing a comparative analysis with human breast cancer. Tumour samples were collected from 30 clinical canine cases and evaluated using Fine Needle Aspiration Cytology (FNAC) and histopathology to classify the types of tumours and identify malignancies. Additionally, haematological parameters such as total leukocyte count and differential leukocyte count were analyzed to assess systemic changes in the affected dogs. In parallel, 30 human breast cancer tissue samples, classified according to the WHO guidelines, were studied to highlight similarities and differences between canine and human mammary tumours. The results revealed key cytological and histopathological features that align with human breast cancer types, particularly in invasive forms. Haematological findings in dogs indicated significant alterations, mirroring some patterns observed in human cancer patients. This comparative approach provides insights into the biological behaviour of canine tumours and their relevance as a model for human cancer studies, potentially aiding in the development of more effective diagnostic and treatment strategies for both species. These findings underscore the importance of cross-species oncology research and suggest that further comparative studies could enhance our understanding of tumour pathogenesis and therapeutic approaches in both veterinary and human medicine.

Key words: Canine mammy tumours, Cytology, Haematology, Histopathology, Human breast cancer.

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Introduction

Cancer is one of the most significant health challenges affecting both humans and animals, characterized by the uncontrolled proliferation of abnormal cells that can invade surrounding tissues and metastasize to distant organs. Among domestic animals, canine tumours are of particular interest due to the increasing incidence and their relevance as models for studying human cancers. Specifically, canine mammary tumours, which account for nearly half of all tumours in female dogs, share many clinical, pathological, and biological features with human breast cancer, making them a valuable model for comparative oncology (Chun *et al.,* 2007). Canine mammary tumours (CMTs) are of particular importance due to their high prevalence and diverse histological subtypes. These tumours can range from benign growths such as fibro adenomas to highly malignant carcinomas with metastatic potential. Studies have shown that certain subtypes of canine mammary carcinomas bear a striking resemblance to human breast cancer in terms of hormonal receptor status, molecular markers, and metastatic behaviour (Goldschmidt *et al.,* 2011). This similarity underlines the importance of studying canine tumours as a comparative model to improve our understanding of cancer biology and treatment in both species. In humans, breast cancer is the most commonly diagnosed cancer in women worldwide, accounting for approximately 25% of all cancer cases in females (Torre *et al.,* 2015). The World Health Organization (WHO) has classified human breast tumours into various subtypes based on histological and molecular features,

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including ductal carcinoma *in situ*, invasive ductal carcinoma, and invasive lobular carcinoma (Tavassoli and Devilee, 2003). This classification is critical for determining prognosis and guiding treatment strategies. Similarly, a histopathological classification system exists for canine mammary tumours, helping clinicians determine the nature and progression of the disease in affected animals (Misdorp, 2002). The study of cancer across species has gained increasing attention in recent years as researchers recognize the benefits of using naturally occurring tumours in animals to model human cancers. The comparative study of canine tumours alongside human breast cancer offers a unique opportunity to investigate shared molecular pathways, treatment responses, and diagnostic techniques, ultimately contributing to the development of better therapeutic strategies for both humans and dogs (Paoloni *et al.,* 2009). This study aims to provide a comprehensive cytological, histopathological, and haematological evaluation of canine tumours, with a focus on mammary tumours, and to compare these findings with human breast cancer.

Materials and Methods

The study was conducted on 30 cases of canine tumours. Fifteen samples were collected from Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, NDVSU, Mhow, and the remaining 15 from private clinics of Indore and Mhow during April 2023 to March 2024. Normal tissue from one cancer-free female dog was also collected as a control. Additionally, 30 human breast tissue slides from 15 cases, prepared by Indore Cancer Foundation Charitable Trust and Sri Aurobindo Institute of Medical Science, were procured for comparison. All chemicals and biologicals used in the present study were of molecular and analytical grade procured from reputed international and national firms, including Sigma (USA), Merck (India), ThermoFisher, Invitrogen, CDH, and Hi-media (India).

Clinical history including breed, age, sex, reproductive status (intact or spayed), tumour recurrence, anatomical location, tumour size, and the number of affected mammary glands was recorded at the time of presentation, including weight of surgically removed tumours. Tumour masses were fixed in 10% neutral buffered formalin and processed for histopathological examination.

Blood samples (2 mL) were collected from the cephalic or saphenous veins of the 30 CMT affected dogs using a 22G needle and transferred immediately to EDTA-coated vials (2 mg/mL blood). Haematological parameters such as total leukocyte count and differential leukocyte count were analyzed following standard procedures (Jain, 1986). Cytological specimens were obtained by fine needle aspiration preoperatively or by preparation of impression smears from excised tumours. At least three slides per tumour were prepared by slide-over-slide smears (squash preparations) or impression smear technique, fixed in absolute methanol, and stained using Giemsa. Tumours were diagnosed based on general criteria including anisocytosis, pleomorphism, and hypercellularity, as well as nuclear criteria such as macrokaryosis, anisokaryosis, multinucleation, nuclear molding, mitotic figures, chromatin pattern, and macronucleoli.

Formalin-fixed, paraffin-embedded tissues were sectioned at 5 µm thickness using a semi-automated microtome. Sections were floated on a warm water bath (50-52°C) containing gelatin to facilitate adherence to slides without wrinkles. Tumours were classified as benign or malignant according to histopathologic criteria proposed by Goldschmidt *et al*. (2011), with sub-classification based on cellular composition, growth patterns, and invasiveness. Canine mammary carcinomas were graded into three categories (I, II, or III) following Pena *et al*. (2013), using tubular structure formation, nuclear pleomorphism, and mitotic index as criteria. Human breast tumours were similarly classified into benign and malignant categories based on the WHO classification system by Tavassoli and Devilee (2003).

The incidence of different types of canine tumours was calculated using the formula:

$$\text{Percentage of incidence} = \frac{\text{Total No. of a particular type tumours found}}{\text{Total number of different cases of tumour}} \times 100$$

Haematological data were analyzed for mean, standard error, and significance using a completely randomized design.

Results and Discussion

Among 30 canine mammary tumour (CMT) cases as detailed in Table 1, 25 (83.3%) were classified as malignant and 5 (16.7%) as benign. Amongst 13 breeds, the Labrador Retriever (30%, 9 cases), non-descript (16.7%, 5 cases), and German Shepherd (13.3%, 4 cases) were recorded as most affected breeds. Other breeds included Pomeranian (3 cases), Pug, Beagle, Dalmatian, Shih Tzu, Dachshund, Alsatian, Golden Retriever, Saint Bernard, and Doberman (one case each). Thus, Labrador Retriever, non-descript, and German Shepherds showed positivity when compared with other breeds of dogs. Breed predisposition corresponded to the regional dog population distribution in Mhow and Indore. The mean age at surgery was 7.93 ± 1.68 years (range 6-11 years), with benign tumours averaging 7.20 ± 1.30 years and malignant tumours 8.08 ± 1.73 years. Incidence of benign tumours peaked between 6-9 years, while malignancies predominated in dogs aged 6-11 years, indicating increased malignancy risk with age. All cases were female, consistent with established literature; male CMTs remain rare. These findings aligned with previous reports associating breed, age, and sex as significant risk factors for CMT occurrence (Gupta *et al.*, 2012; Raval, 2017).

Tumour localization (Table 1) showed the highest incidence in the right caudal abdominal (7 cases), right inguinal (6), left caudal abdominal (5), and left inguinal (4) mammary glands. Lesser occurrences were noted in right cranial and right caudal thoracic glands (2 each), with single

Table 1: Clinical history of cases (intact female dogs) showing incidence of canine mammary tumours (n=30)

Case No.	Breed	Age (years)	Tumour size (cm)	Tumour weight (g)	Location	No. of glands	Consistency	Surface ulceration
1.	German Shepherd	7	9-10	220	Right inguinal	1	Round and Hard	Yes
2.	Labrador	8	8-9	480	Bilateral caudal inguinal	2	Round and Soft	No
3.	Pug	10	4-5	320	Left inguinal	1	Round and Hard	Yes
4.	Pomeranian	11	4-5	150	Right inguinal	1	Round and Soft	No
5.	Dachshund	10	4-5	140	Right cranial thoracic	1	Multilobulated & Hard	No
6.	Non descript	6	8-9	230	Right caudal abdominal	1	Irregular and Soft	Yes
7.	Golden Retriever	7	8-9	230	Right caudal abdominal	1	Round and Hard	No
8.	Saint Bernard	6	10-11	650	Left caudal abdominal	1	Irregular and Hard	No
9.	Non descript	9	7	765	Right cranial thoracic	1	Irregular and Hard	No
10.	Beagle	7	4-5	260	Left caudal abdominal	1	Round and Soft	No
11.	Labrador	11	7	490	Right caudal abdominal	1	Round and Hard	No
12.	Alsatian	8	6	275	Left inguinal	1	Irregular and Hard	Yes
13.	Doberman	7	6	326	Right inguinal	1	Irregular and Hard	No
14.	German Shepherd	9	6	241	Bilateral caudal abdominal	2	Round and Hard	No
15.	Non descript	6	5	630	Right caudal abdominal	1	Round and Hard	No
16.	Dalmatian	8	5	450	Right inguinal	1	Round and Hard	No
17.	Labrador	7	4-5	256	Right caudal thoracic	1	Irregular and Hard	No
18.	Labrador	6	5	350	Left inguinal	1	Round and Hard	Yes
19.	German Shepherd	11	7	285	Left inguinal	1	Multilobulated &Hard	No
20.	Labrador	10	6	220	Right inguinal	1	Oval and Hard	No
21.	Labrador	8	2	630	Right caudal abdominal	1	Round and Hard	Yes
22.	Pomeranian	7	3-4	250	Left caudal abdominal	1	Multilobulated & Hard	Yes
23.	Labrador	10	5	168	Left caudal abdominal	2	Round and Hard	No
24.	German Shepherd	7	6	316	Left caudal thoracic	1	Irregular and Hard	Yes
25.	Non descript	6	3	892	Right inguinal	1	Irregular and Hard	No
26.	Labrador	8	4	114	Left caudal abdominal	1	Round and Soft	No
27.	Shihtzu	6	4-5	110	Left cranial abdominal	1	Round and Soft	No
28.	Pomeranian	9	4	148	Right caudal thoracic	1	Round and Soft	No
29.	Labrador	6	4	138	Right caudal abdominal	1	Round and Soft	No
30.	Non descript	7	4	148	Right caudal abdominal	1	Irregular and Hard	Yes

cases in bilateral caudal inguinal, bilateral caudal abdominal, left caudal thoracic, and left cranial abdominal locations. Laterally, tumours predominantly involved the right side (17 cases), followed by the left (11) and bilateral (2). These observations concurred with previous studies reporting the inguinal mammary glands as the most frequently affected (Acharya *et al.,* 2017; Raval, 2017).

Gross examination revealed varied tumour morphologies: round and hard (10), irregular and hard (8), round and soft (7), multilobulated and hard (3), with one case each of irregular and soft, and oval and hard tumours. Tumour weights ranged from 110 g to 892 g. Surface ulcers were observed in nine tumours (Table 1). These findings were comparable to prior reports noting diverse tumour consistencies and weights (Baba *et al.,* 2016; Acharya *et al.,* 2017).

Cytological analysis identified anisocytosis, anisokaryosis, hypercellularity, multinucleation, and mitotic figures in malignant tumours (Fig. 1), whereas benign tumours exhibited cellular uniformity, increased nuclear-to-cytoplasmic ratio, and occasional anisonucleosis. Based on these criteria, 25 tumours were classified as malignant and 5 as benign, which is consistent with results of earlier studies (Haziroglu *et al.,* 2010).

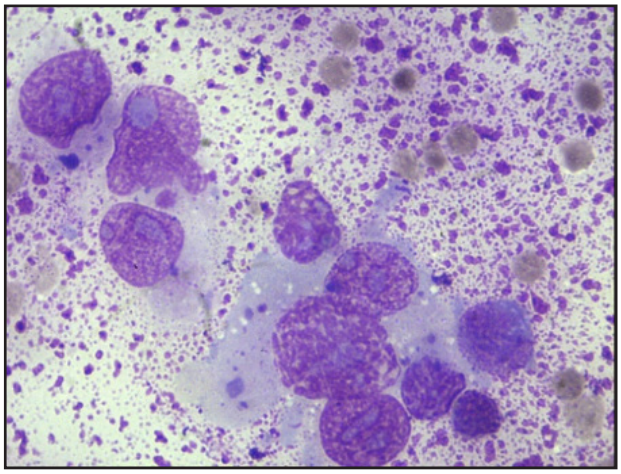

Fig. 1: Loose clusters of hyperchromatic pleomorphic cells with vacuolated nucleus, an irregular nuclear membrane and clear narrow rim of cytoplasm in a case of carcinosarcoma (Giemsa X 1000)

Haematological comparison revealed a significantly higher total leukocyte count in malignant tumours (20.00 ± 1.41 $\times 10^3$/µL) compared to benign tumours (14.22 ± 0.37 $\times 10^3$/µL). No significant differences were observed in differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils). These findings (Table 2) aligned with previously published data (Kumar *et al.,* 2018; Munsef *et al.,* 2018).

Table 2: Haematological parameters in benign and malignant mammary tumours (n=30)

Parameters	Normal range	Benign	Malignant
TLC(x10^3/µL)	6-17 (11.5)	14.22 ± 0.37	20.00 ± 1.41*
Neutrophil (%)	60-77 (68.5)	76.88 ± 1.26	73.93 ± 2.77
Lymphocyte (%)	12-30 (21)	15.80 ± 1.19	19.82 ± 2.83
Monocyte (%)	3-10 (6.5)	3.24 ± 0.31	3.68 ± 0.46
Eosinophil (%)	2-10 (6.0)	3.10 ± 0.76	1.66 ± 0.37
Basophil (%)	0-2 (0.7)	1.00 ± 0.18	0.81 ± 0.14

*Significant between types at 5% level.

Table 3: Histopathological classification of canine mammary neoplasms (n=30)

Type of Proliferation	No.	%
Benign Neoplasms		
Adenoma simple	3	10.00
Fibroadenoma	2	6.66
Total Benign cases	**5**	**16.66**
Malignant Epithelial Neoplasms		
Carcinoma tubular	6	20.00
Carcinoma *in situ*	1	3.33
Carcinoma micropapillary invasive	1	3.33
Carcinoma complex type	5	16.66
Carcinoma and malignant myoepithelioma	1	3.33
Carcinoma arising in complex adenoma/ mixed tumour	1	3.33
Ductal carcinoma	1	3.33
Malignant Epithelial Neoplasms: Special Type		
Lipid rich secretory carcinoma	2	6.66
Carcinosarcoma	4	13.33
Malignant Mesenchymal Neoplasm-Sarcoma		
Fibrosarcoma	2	6.66
Hemangiosarcoma	1	3.33
Total Malignant cases	**25**	**83.33**
Grand Total	**30**	**--**

Among 30 CMT cases, 83.3% (25) were of malignant type and 16.7% (5) were of benign type. Benign tumours comprised mainly adenomas (10%) and fibroadenomas (6.7%), while malignant tumours included malignant epithelial neoplasms (53.3%), malignant mixed tumours (13.3%), and mesenchymal neoplasms (10%). Tubular carcinoma (20%) and complex carcinoma (16.7%) were the most frequent malignant types (Table 3), which is consistent with the prior studies (Reddy *et al.,* 2009; Acharya *et al.,* 2017).

Histopathologically, benign adenomas were non-infiltrative with minimal atypia; fibroadenomas showed multilobulated tubuloacinar structures (Fig. 2). Malignant tumours displayed varied features: tubular and complex carcinomas showed pleomorphism and invasiveness; carcinosarcomas contained mixed malignant epithelial and connective tissue cells (Fig. 3); haemangiosarcomas had pleomorphic endothelial cells forming immature vessels, classified as per WHO and Goldschmidt *et al.* (2011) criteria.

In this study, 25 canine mammary carcinomas (CMCs) were histopathologically graded using the Pena *et al.* (2013) modification of the Elston and Ellis (Nottingham) grading system, which assesses tubule formation, nuclear pleomorphism, and mitotic figures per 10 high-power fields. Tumours were classified as grade I (well-differentiated, 56%), grade II (moderately differentiated, 32%), and grade III (poorly differentiated, 12%). Grade I tumours demonstrated >75% tubule formation, minimal nuclear atypia, and low mitotic activity, whereas grade III tumours had <10% tubule formation, marked nuclear pleomorphism, and high

mitotic counts. This grading distribution reflects tumour heterogeneity and aligns with prior studies reporting similar histopathological variations. Carcinoma subtypes including tubular carcinoma, complex carcinoma (Fig. 4), and carcinosarcoma (Fig. 5) were represented across all grades, underscoring diverse tumour behaviour within CMCs (Table 4).

In this study, additional histopathological changes observed in 25 malignant canine mammary tumours (CMTs) included mild to moderate inflammation (21 cases), connective tissue invasion (20), epithelial-mesenchymal transition (EMT) and necrosis (15 each), tumour thromboembolism (12), tumour-infiltrating lymphocytes (TIL) (10) (Fig. 6), and unclear tumour margins (20). These features, although not widely documented in CMTs, parallel patterns seen in human breast cancer. EMT and necrosis, both linked to poor prognosis, were notable, as was the high prevalence of inflammation. Unlike in human breast carcinoma, where tumours are distinctly classified as inflammatory or non-inflammatory, such subclassification in veterinary oncology remains undefined.

Thirty human breast tumours (HBTs) were classified as malignant (66.7%, 20 cases) or benign (33.3%, 10 cases). Invasive ductal carcinoma (Fig. 7) was the most common malignant type (43.3%, 13 cases), followed by ductal carcinoma *in situ* (Fig. 8) (23.3%, 7 cases). Among benign tumours, fibroadenoma predominated (30%, 9 cases) with one case (3.3%) of phyllodes tumour. Malignant tumours exhibited features such as perivascular invasion and hyperchromatic nuclei, whereas benign fibroadenomas showed well-circumscribed biphasic growth. Phyllodes tumours displayed characteristic leaf-like epithelial structures and myxoid stromal proliferation (Table 5)

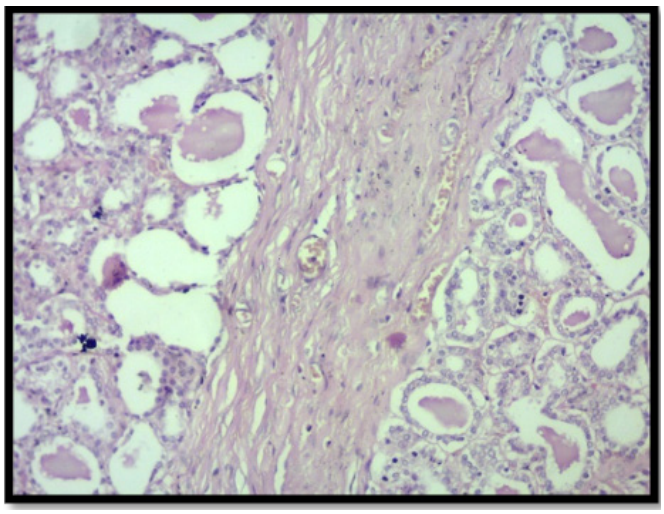

Fig. 2: Simple adenoma: Showing thickening of interstitium around alveoli and ducts with presence of multiple corpora amylacea (H&E X 100)

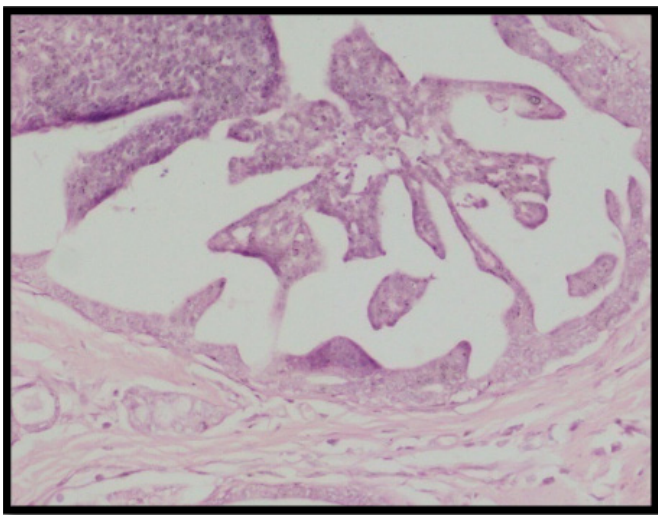

Fig. 3: Carcinoma *In situ*: Neoplastic cells forms tubules with papillae extending into their lumina supported by fine fibrovascular connective tissue stroma (H&E X 200)

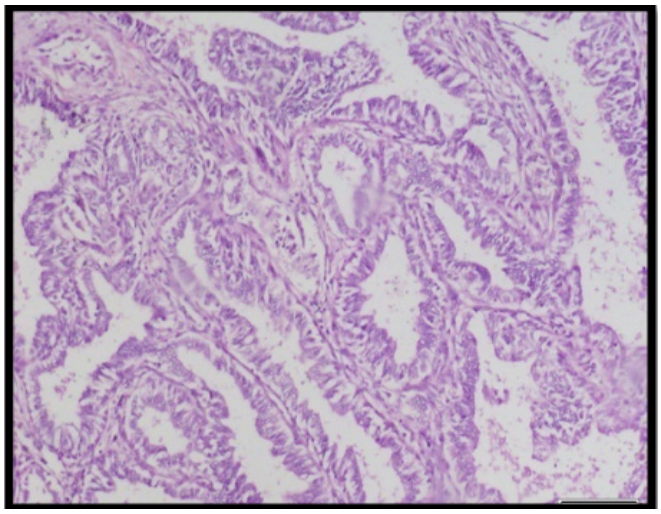

Fig. 4: Carcinoma complex type: Carcinogenic myoepithelial cells showing lacelike pattern of arrangement with frilled edges (H&E X 100)

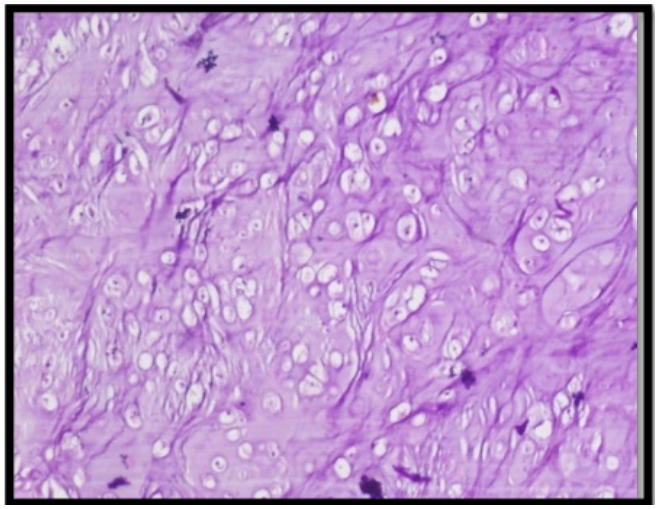

Fig.5: Carcinosarcoma: Chondroblasts within chondroid matrix with presence of mitotic figures and pleomorphism (H&E X 200)

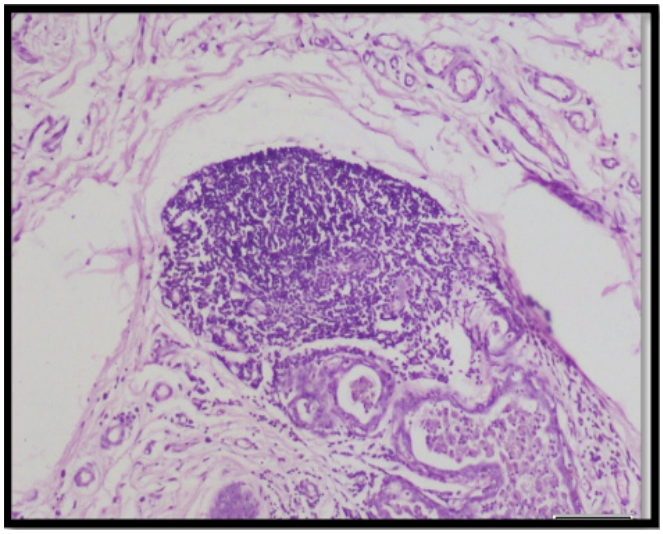

Fig. 6: Carcinoma tubular: Tumour infiltrating lymphocyte (TIL) massive infiltration of lymphocytes and extensive tubule formation (H&E X 200)

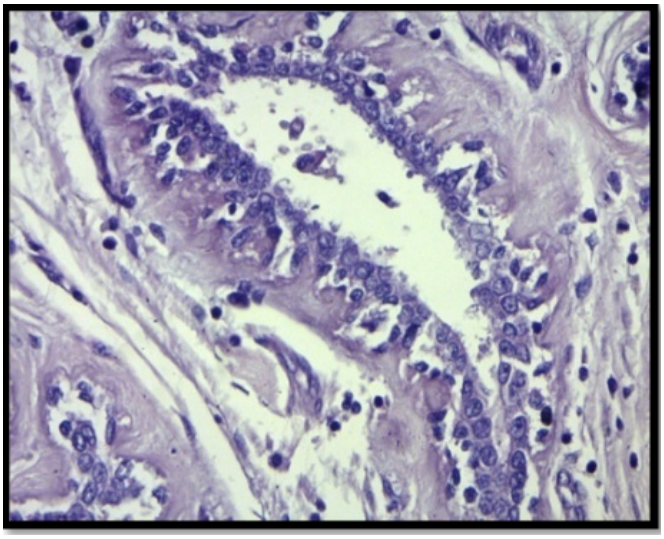

Fig. 7: Invasive ductal carcinoma of breast: Poorly differentiated invasive carcinoma of special type ragged sheets of pleomorphic cells without tubule formation infiltrate into adjacent stroma (H&E X 200)

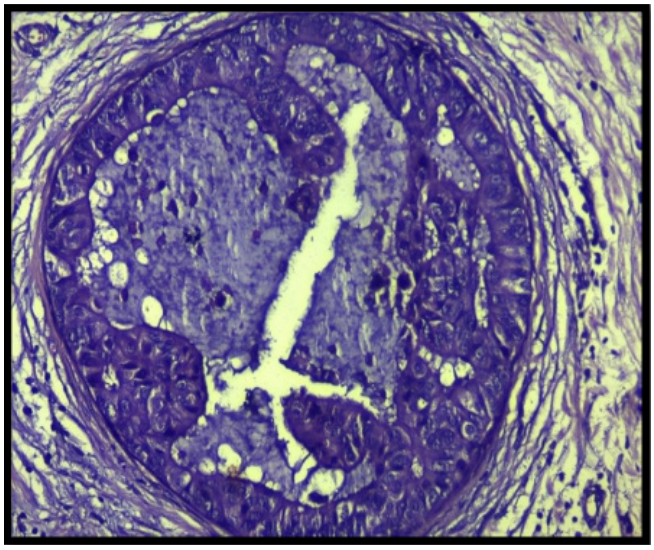

Fig. 8: Ductal carcinoma *in situ* of breast: Proliferation of neoplastic cells into the ductal lumen without invasion of basement membrane (H&E X 200)

Histopathological grading of human breast carcinomas (HBCs) was performed using the Elston and Ellis (Nottingham) system, evaluating tubule formation, nuclear pleomorphism, and mitotic figures per 10 high-power fields. Among 13 invasive ductal carcinomas, 2 were of grade I, 7 of grade II, and 4 of grade III. Of 7 ductal carcinomas *in situ* cases, 4 were of grade II and 3 of grade III. Overall, 10% cases were of grade I (≥75% tubule formation, small uniform nuclei, few mitoses), 55% of grade II (10-75% tubule formation, moderate nuclear atypia, moderate mitoses), and 35% of grade III (<10% tubule formation, marked nuclear variation, hyperchromatic nuclei, numerous mitoses) (Table 6).

Table 4: Histopathological grading of canine mammary carcinomas (n=25)

Histopathological type	No.	Histopathological Grade		
		Grade I	Grade II	Grade III
Carcinoma tubular	6	2	2	2
Carcinoma *in situ*	1	1	-	-
Carcinoma micro-papillary invasive	1	1	-	-
Carcinoma Complex type	5	2	2	1
Carcinoma & malignant myoepithelioma	1	1	-	-
Carcinoma arising in complex adenoma/ mixed tumour	1	1	-	-
Ductal carcinoma	1	-	1	-
Lipid rich secretory carcinoma	2	1	1	-
Carcinosarcoma	4	2	2	-
Fibrosarcoma	2	2	-	-
Hemangiosarcoma	1	1	-	-
Total	**25**	**14 (56%)**	**8 (32%)**	**3 (12%)**

Table 5: Histopathological classification of human breast tumours (n=30)

Histopathological Type	Malignancy	No.	%
Invasive ductal carcinoma	Malignant	13	43.34
Ductal carcinoma *in situ*	Malignant	7	23.33
Fibroadenoma	Benign	9	30.00
Benign phyllodes	Benign	1	3.33

Table 6: Histopathological grading of human breast cancer (n=20)

Histopathological Type	No.	Histopathological Grade		
		Grade I	Grade II	Grade III
Invasive ductal carcinoma	13	2	7	4
Ductal carcinoma *in situ*	7	-	4	3
Total	**20**	**2(10%)**	**11(55%)**	**7(35%)**

Conclusion

The present comparative study of cytological, histopathological, and haematological evaluation of canine tumours and human breast cancer provides valuable insights into tumour biology across species. Canine mammary tumours predominantly affected older, non-spayed bitches aged 6-11 years, with Labrador Retrievers exhibiting the highest incidence, supporting ovario-hysterectomy as a preventive measure. FNAC and impression smears proved effective for rapid, minimally invasive diagnosis in both species. Leukocytosis was commonly observed in malignant tumours, reflecting systemic responses and underscoring its diagnostic utility. Malignant neoplasms, especially malignant epithelial tumours, were more prevalent than benign types, with simple adenomas and fibroadenomas predominant among benign lesions. Canine mammary carcinomas (CMCs) were graded as 56% of grade I, 32% of grade II, and 12% of grade III. Histopathological analyses revealed tumour parallels such as adenocarcinomas and invasive ductal carcinomas in both dogs and humans. The incidence and types of canine mammary tumours closely mirror human breast cancer patterns, reinforcing the dog's role as a comparative oncology model for animal welfare and advancing human cancer biology. Future molecular research promises improved diagnostics and therapeutics benefiting both veterinary and human medicine.

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