RESEARCH ARTICLE

Application of Immunohistochemistry in Tumour Classification: A Comparative Study of Canine and Human Tissues

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Abstract

This study was aimed to perform a comparative analysis of canine mammary tumours (CMT) and human breast cancer (HBC) by evaluating similarities in molecular characteristics and assessing the transmodel potential of CMT for human breast cancer research. Immuno-histochemistry (IHC) was employed to analyze the expression of key biomarkers, *viz.,* estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and Ki-67 in both canine and human mammary tumour tissues (n=30 each). Tissue samples were processed using standard IHC protocols, including antigen retrieval, blocking of endogenous peroxidase, and incubation with primary and secondary antibodies. Biomarker expression levels were quantified and compared across tumour subtypes in both species. The analysis revealed significant parallels in the expression patterns of ER, PR, HER-2, and Ki-67 between CMT and HBC. Molecular subtypes identified in canines, including luminal A, luminal B, HER-2 overexpressing, and triple-negative, closely mirrored those classified in human breast cancer. Furthermore, the proliferation marker Ki-67 exhibited comparable expression across species, suggesting similar tumour growth dynamics. These findings demonstrate that canine mammary tumours share molecular and histological characteristics with human breast cancer, supporting their value as a translatable model in breast cancer research. Consequently, canine mammary tumours represent a valuable platform for investigating tumour biology and therapeutic interventions relevant to human breast cancer.

Key words: Canine mammary tumours, Comparative oncology, Human breast cancer, Immunohistochemistry, Molecular subtypes.

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Introduction

Canine mammary tumours (CMTs) are among the most common neoplasms in female dogs, comprising nearly 50% of tumours in intact females over 7 years old. Their clinical behaviour varies widely from benign to highly aggressive malignant forms, often metastasizing and reducing survival (Dutra *et al*., 2004). This variability has prompted the search for prognostic biomarkers - measurable indicators of disease presence and severity - similar to those used in human breast cancer (Goldschmidt *et al*., 2011; Pena *et al*., 2014). Key biomarkers in CMTs include hormone receptors (estrogen receptor ER, progesterone receptor PR), proliferation markers (Ki-67), and growth factors (HER2/neu), which predict tumour behaviour, metastatic potential, and survival outcomes (Sorenmo *et al*., 2013). These biomarkers mirror those in human breast cancer, supporting CMTs as a valuable translational model (Valdivia *et al*., 2021). ER and PR positivity generally associates with better tumour differentiation and prognosis (Millanta *et al*., 2005), whereas HER2/neu overexpression correlates with poor prognosis and higher metastasis. Elevated Ki-67 levels indicate more aggressive tumours and shorter survival (Kim *et al*., 2005; Abdelmegeed and Mohammed, 2018).

Despite growing knowledge, prognostic significance of biomarkers in CMTs requires further study due to breed differences, histological subtypes, and molecular variability among dogs (Vazquez *et al*., 2023). Conflicting findings highlight the complexity of tumour biology. Integrating biomarker research in CMTs promises advances in veterinary oncology and comparative oncology, potentially improving human cancer therapies. Using CMTs as a comparative model may aid in discovering new biomarkers and treatment targets relevant across species. This study was aimed to evaluate the prognostic value of hormone receptors, growth factors, and proliferation markers in CMTs

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to guide clinical decisions and predict tumour progression, ultimately supporting personalized and effective veterinary cancer therapies.

Materials and Methods

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

Evaluation of Molecular Biomarker Expression in Tissues by Immunohistochemistry

Immunohistochemistry (IHC) was performed to assess the expression of biomarkers in mammary tumour samples and classify them into molecular subtypes as per standard protocol described by Pena *et al*. (2014). The primary antibodies used for IHC of canine mammary tumours included rabbit anti-ER-α (Invitrogen; HRPO detection; monoclonal; 1:50), rabbit anti-PR (Invitrogen; HRPO detection; monoclonal; 1:100), mouse anti-HER-2 (Invitrogen; avidin–biotin peroxidase detection; monoclonal; 1:400), and mouse anti-Ki-67 (Invitrogen; HRPO detection; monoclonal; 1:100).

Evaluation System for HER-2

Interpretation of HER-2 immunostaining was carried out as per American Society of Clinical Oncology (ASCO) guidelines suggested by Ahn *et al*. (2020). The scoring criteria for HER-2 immunostaining was defined as weak to strong full membrane staining of more than 10% of cancer cells.

Ki67 Proliferation Index

The positive reaction for Ki-67 proliferation antigen was observed as dark brown coloured granular material restricted to the nucleus of proliferating cells. Ki-67 expressions were mainly assessed at the periphery of the tumour where the active proliferation is likely to be higher than in other areas as suggested by Raval (2017).

Molecular Classification of CMCs

Canine mammary carcinomas (CMCs) were classified in five subtypes according to the expression of ER, PR, HER-2 and Ki-67 as proposed by Gamma *et al*. (2008) (Table 1).

Table 1: Molecular classification of CMCs (Gamma *et al.,* 2008)

Subtypes	Molecular Signature
Luminal like A	ER+, PR±, HER2-, Low/High Ki 67
Luminal like B	ER+, PR±, HER2+, Low/High Ki 67
HER-2 Over expression	ER-, PR-, HER2+, Low/High Ki 67
Basal like (Triple negative)	ER-, PR-, HER-, High Ki 67
Unclassified or Normal like	Negative for all markers

Classification of Human Breast Tumour Cases

A total of 30 human breast tumour cases were classified into benign and malignant categories based on the WHO classification system adopted by Tavassoli and Devilee (2003). The classification includes non-invasive tumours such as ductal carcinoma *in situ*, intraductal papillary carcinoma, and lobular carcinoma *in situ*. Invasive tumours identified in the study included invasive ductal and lobular carcinomas, medullary carcinoma, colloid carcinoma, Paget's disease, tubular carcinoma, adenoid cystic carcinoma, invasive comedocarcinoma, apocrine carcinoma, and invasive papillary carcinoma. Benign tumours classified included fibroadenoma, phyllodes tumour, and intraductal papilloma.

Results and Discussion

Immunohistochemistry and Molecular Subtypes of CMCs

In both human and veterinary medicine, immunohistochemistry (IHC) is crucial for identifying neoplasms and prognostic factors that can help target therapies and improve survival rates (Leong *et al.,* 2010; Queiroga *et al.,* 2011). In this study, 25 canine mammary carcinomas (CMCs) were similarly classified into these subtypes according to the expression of ER, PR, HER-2, and Ki-67, following the criteria of Gama *et al.* (2008). Tumours displayed varying levels of immunoreactivity. Tubular carcinomas (n=6) showed variable ER/PR positivity with frequent strong HER-2 (++/+++) expression and occasional Ki-67 positivity (Fig. 1). Carcinoma *in situ* (n=1) was HER-2 positive (++), while micropapillary invasive carcinoma (n=1) showed ER and PR positivity without HER-2 or Ki-67. Complex type carcinomas (n=5) generally expressed ER/PR with strong HER-2 (++/+++) and occasional Ki-67 positivity. A carcinoma with malignant myoepithelioma was negative for ER, PR, HER-2 but Ki-67 positive. Carcinoma arising in a mixed tumour expressed ER and HER-2 (++) but lacked PR and Ki-67. Ductal carcinoma showed ER and HER-2 positivity (Fig. 2). Lipid-rich secretory carcinomas (n=2) expressed ER, with variable PR and HER-2 positivity, and one showed Ki-67 (Fig. 3). Carcinosarcomas (n=4) displayed heterogeneous ER/PR expression (Fig. 4) with occasional HER-2 and Ki-67 positivity. Fibrosarcomas (n=2) and hemangiosarcoma (n=1) were negative for ER, PR, and HER-2 but consistently Ki-67 positive.

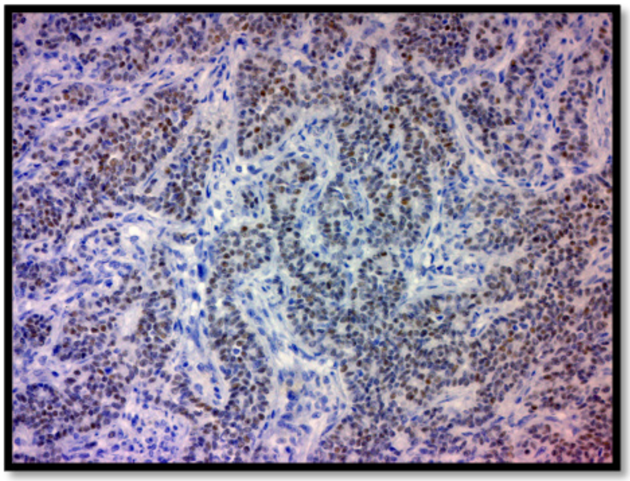

Fig. 1: Carcinoma tubular: Neoplastic cells showing moderate nuclear immune-reactivity with anti ER antibody, Immunoperoxidase staining (DAB X100)

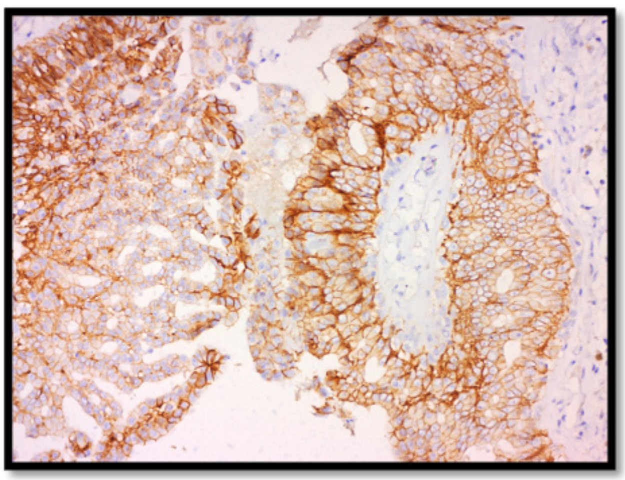

Fig. 2: Ductal carcinoma: HER-2 overexpression, neoplastic cells showing diffuse incomplete, moderate circumferential membrane staining, Immunoperoxidase staining (DAB X200)

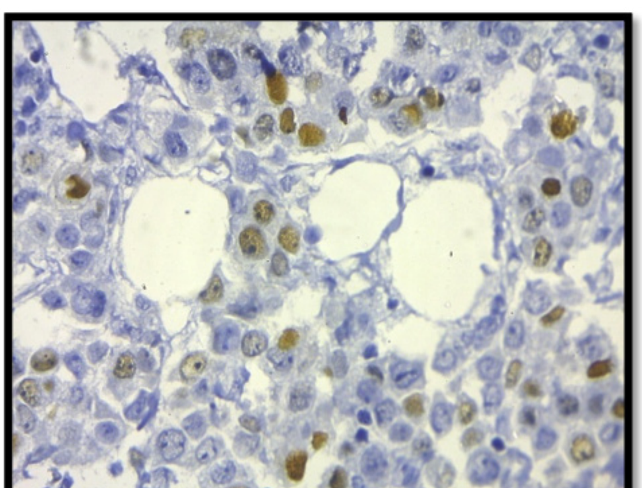

Fig. 3: Lipid rich secretory carcinoma: Neoplastic cells showing weak nuclear immunoreactivity with Ki-67 antibody, Immunoperoxidase staining (DAB X400)

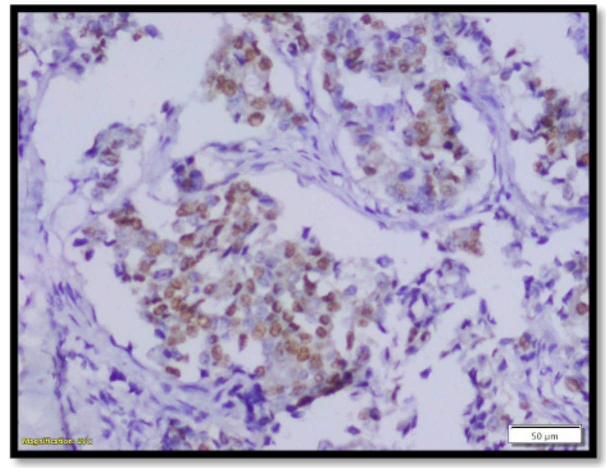

Fig. 4: Carcinosarcoma: Neoplastic cells showing moderate nuclear immunoreactivity with anti-PR antibody, Immunoperoxidase staining (DAB X200)

Out of 25 canine mammary carcinomas (CMCs) examined, luminal B was the most frequent subtype (9/25, 36%), followed by HER-2 overexpression (7/25, 28%), luminal A (5/25, 20%), and basal-like (4/25, 16%), with no normal-like cases was identified (Table 2). Among tubular carcinomas (n=6), two were luminal A, one luminal B, and three HER-2 overexpression. Carcinoma in situ (n=1) was classified as HER-2 overexpression, while micropapillary invasive carcinoma (n=1) fell under luminal A. Of the five complex carcinomas, two were luminal B and three HER-2 overexpression. Carcinoma with malignant myoepithelioma (n=1) was basal-like, whereas carcinoma arising in a complex adenoma/mixed tumour (n=1) and ductal carcinoma scirrhous adenocarcinoma (n=1) were both luminal B. Lipid-rich secretory carcinomas (n=2) were also luminal B. Carcinosarcomas (n=4) were split, with two luminal A and two luminal B. Fibrosarcomas (n=2) and hemangiosarcoma (n=1) were basal-like.

These findings deferred from previous reports. Ribeiro *et al.* (2012) observed a predominance of luminal A (41.4%), followed by basal-like (27.6%), HER-2 (17.2%), and luminal B (13.8%). Im *et al.* (2014) reported luminal A as most common (70 cases), followed by basal-like (39), luminal B (36), HER-2 (9), and normal-like (5). Shinoda *et al.* (2014) noted basal-like as predominant (33%), followed by luminal A (25%), luminal B (23%), HER-2 overexpressing (15%), and normal-like (4%). Sassi *et al.* (2010) reported luminal B as the most frequent, followed by luminal A and basal-like, with no HER-2 overexpression. Raval (2017) found basal-like to be most common (50.85%), followed by luminal A (20.34%), HER-2 overexpression (11.86%), luminal B (10.17%), and normal-like (6.78%).

Immunohistochemistry Expression of Human Breast Carcinomas

In human ductal carcinoma *in situ* (DCIS), the majority of cases demonstrated strong ER (often +++) and PR (++,

Table 2: Association between molecular subtype and histopathological classification (n=25)

Histopathological type	No. of case	Molecular subtype				
		Luminal A	**Luminal B**	**HER2 overexpression**	**Basal like**	**Normal like**
Carcinoma tubular	6	2	1	3		
Carcinoma *in situ*	1			1		
Carcinoma micropapillary invasive	1	1				
Carcinoma complex type	5		2	3		
Carcinoma and malignant myoepithelioma	1				1	
Carcinoma arising in Complex adenoma/ Mixed tumour	1		1			
Ductal carcinoma	1		1			
Lipid rich secretory carcinoma	2		2			
Carcinosarcoma	4	2	2			
Fibrosarcoma	2				2	
Hemangiosarcoma	1				1	
Total	**25**	**5 (20%)**	**9 (36%)**	**7 (28%)**	**4 (16%)**	

+++) positivity (Fig. 5), with HER-2 largely negative but occasionally weakly positive. Ki-67 expression was mostly low (+) to moderate (++), suggesting limited proliferative activity. One exceptional case was completely negative for ER, PR, HER-2, and Ki-67, representing a triple-negative phenotype. Overall, DCIS cases were dominated by hormone receptor–positive, low-proliferative tumours, consistent with luminal subtypes.

In invasive ductal carcinoma (IDC), expression patterns were more heterogeneous. Several cases were HER-2 overexpressed (+++) with moderate Ki-67 (++), indicating an aggressive phenotype (Fig. 6). A notable proportion were triple-negative (ER–, PR–, HER-2–) with high Ki-67 (++), reflecting basal-like features. Some cases retained strong ER/PR positivity without HER-2, with low to moderate Ki-67, resembling luminal subtypes. Thus, IDC showed a broader immunoprofile distribution, including luminal, HER-2 overexpression, and basal-like subtypes, with higher proliferative activity compared to DCIS.

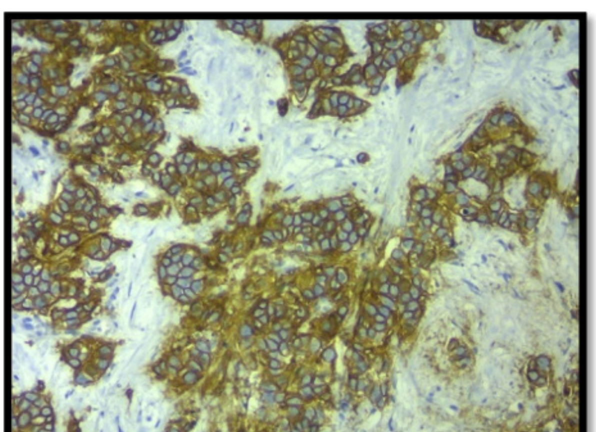

Fig. 5: Ductal carcinoma *in situ* of breast: Neoplastic cells showing intense nuclear immunoreactivity with anti-PR antibody, Immunoperoxidase staining (DAB X400)

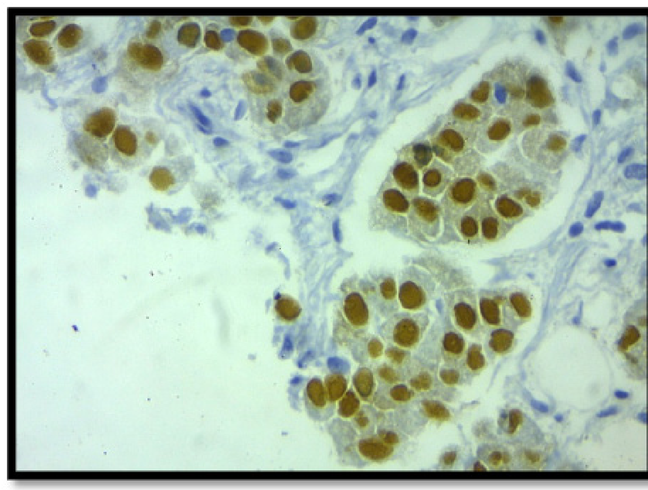

Fig. 6: Invasive ductal carcinoma of breast: HER-2 overexpression. Neoplastic cells showing diffuse complete, intense circumferential membrane staining. Immuno-peroxidase staining (DAB X400)

Molecular Classification of Human Breast Carcinoma

In the present study, 20 human breast carcinomas, comprising 13 invasive ductal carcinomas (IDC) and 7 ductal carcinoma *in situ* (DCIS), were classified into five molecular subtypes based on ER, PR, HER-2, and Ki-67 expression. Among the IDC cases, three were categorised as Luminal A, five as HER-2 overexpression, and five as basal-like (triple negative). Within the DCIS group, four cases were Luminal A, two Luminal B, and one normal-like. Overall, Luminal A was the most common subtype (7/20, 35%), followed by HER-2 overexpression and basal-like, each accounting for 25% (5/20), while Luminal B (2/20, 10%) and normal-like (1/20, 5%) were less frequent (Table 3).

Immunohistochemical studies of breast cancer began in the 1980s (Hsu *et al.,* 1981), leading to the identification of many glycoprotein markers essential for assessing tumour

Table 3: Association with molecular sub type and histopathological classification (n=20)

Histopathological type	No. of case	Luminal A	Luminal B	HER2 overexpression	Basal like (Triple Negative)	Normal like
Invasive ductal carcinoma	13	3		5	5	
Ductal carcinoma *in situ*	7	4	2			1
Total	**20**	**7 (35%)**	**2 (10%)**	**5 (25%)**	**5 (25%)**	**1 (5%)**

presence, aggressiveness, and prognosis (Lichtenbeld *et al.,* 1998). While human mammary tumour research has long relied on mouse models and cell lines, the close biological similarities between dogs and humans have made canines valuable in comparative oncology. Factors such as a homologous genome, genetic diversity, spontaneous tumour development, shared environments, and similar tumourigenic pathways support their use (Hawai *et al.,* 2013; Carvalho *et al.,* 2016; Sultan and Ganaie, 2018). Comparative studies have revealed parallels in mammary tumour morphology and taxonomy (Sorenmo *et al.,* 2009), though further research is needed to resolve uncertainties in tumour behaviour and classification.

In this study, 30 canine mammary tumour specimens were examined, predominantly from breeds predisposed to these tumours (Labrador, Non-Descript, German Shepherd), aged 6-11 years. Malignant tumours predominated (83.33%), mainly tubular carcinomas, while benign cases included adenomas and fibroadenomas. This malignancy rate was higher than previously reported (41-53% or 34% in Beagles, Ribeiro *et al.,* 2012), possibly reflecting late detection, delayed spaying, and underreporting of benign cases. Multicentric tumours were observed in older dogs, suggesting independent primary tumour growth. Mixed histological subtypes were also found, consistent with earlier studies describing transitions between solid, papillary, and other forms.

Microscopically, canine and human tumours showed strong similarities in cell origin, growth patterns, and behaviour. Tubular carcinoma in dogs closely resembled human invasive ductal carcinoma, with duct wall invasion, desmoplastic reactions, and lymphatic spread. Other forms, including mucinous carcinoma, invasive micropapillary carcinoma, and comedo-carcinoma, showed comparable morphology in both species. Mixed mammary tumours in dogs contained epithelial and mesenchymal elements, parallel human metaplastic carcinomas and phyllodes tumours. Differences remain in classification, as WHO taxonomy groups certain forms (solid, cribriform, papillary) under invasive carcinoma of "No Special Type" in humans, while canine taxonomy treats them as independent entities (Goldschmidt *et al.,* 2011).

Immunohistochemical analysis confirmed the presence and similar localization of ER, PR, HER-2, and Ki-67 in both species, consistent with earlier studies (Nowak *et al.,* 2005; Joensuu *et al.,* 2013; Pena *et al.,* 2014; Raval, 2017; Munsef *et al.,* 2018). These markers, widely used in diagnosis and research, highlighted striking parallels in biomarker expression, tumour morphology, and behaviour between canine and human mammary tumours. Collectively, these findings strengthen the argument for dogs as a reliable experimental model for studying human breast cancer.

Conclusion

Comparative analysis highlights strong parallels between canine mammary tumours and human breast cancer in biology, histopathology, and markers (ER, PR, HER-2, Ki-67), supporting their role as reliable translational models. These similarities enable cross-species insights into tumour behaviour, prognosis, and therapy, while differences in anatomy and hormonal factors account for some variations. Despite minor IHC procedural differences, diagnostic and prognostic outcomes remain comparable. Using canine models offers an ethical, practical platform to explore novel diagnostics and treatments, including hormone receptor–based and anti-resistance therapies, advancing both human and veterinary oncology.

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