

Original research article

CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF MAMMARY TUMOURS IN INTACT FEMALE DOGS

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Abstract

Canine mammary tumors (CMTs) are among those most frequently diagnosed in intact female dogs. They share biological and hormonal similarities with human breast cancer, which renders them valuable models in comparative oncology. This study aimed to evaluate the clinical, histopathological, and immunohistochemical characteristics of CMTs in intact female dogs. A total of 22 archived CMT cases submitted between 2013 and 2023 was conducted at the Federal University of Agriculture, Abeokuta. Samples were obtained through mastectomy or nodulectomy from female dogs presented at private veterinary clinics and a teaching hospital. Clinical data, including age, tumour size, location, lymph node involvement, and spay status, were recorded. Most dogs were German Shepherds (45.5%) with a mean age of 8.15 years; none were spayed. The tumours were predominantly malignant (77.3%) and commonly located in the inguinal mammary glands (40.9%). Stage T₂ (45.5%) and histological grade II (72.7%) were the most prevalent. E-cadherin was immunopositive in 89% of tumours, with stronger expression in malignant cases. N-cadherin expression was significantly associated with malignancy ($p = 0.001$), indicating epithelial-mesenchymal transition (EMT). Oestrogen receptors α (OR α) was strongly expressed in all tumours, with significantly higher levels in cases of malignancy ($p = 0.002$). Progesterone receptor (PR) and vimentin were observed only in

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malignant tumours, while Ki-67 showed mild, non-significant expression. These findings suggest that N-cadherin, OR α , and PR may serve as potential biomarkers of malignancy and hormonal responsiveness in CMTs. They also support the relevance of molecular profiling in the diagnosis and management of canine mammary tumours.

Keywords: Canine mammary tumors, comparative oncology, immunohistochemistry, N-cadherin, oestrogen receptors

INTRODUCTION

Mammary gland tumors have become a significant concern in both human and veterinary medicine due to their increasing incidence (Vascellari et al., 2016). In dogs, canine mammary tumors (CMTs) are the second most frequently diagnosed neoplasms after skin tumors (Terzaghi et al., 2020). Studies estimate that CMTs account for 50–70% of all tumors diagnosed in sexually intact female dogs (Sorenmo et al., 2013; Terzaghi et al., 2020).

Three major risk factors have been identified in the development of CMTs: prolonged hormonal exposure, advancing age (particularly in middle-aged and older dogs), and breed predisposition (especially among purebred dogs). Other contributing factors include nutritional influences and genetic susceptibility (Sorenmo et al., 2013).

CMTs are histologically classified as simple (comprising a single cell type—either epithelial or myoepithelial), complex (containing both epithelial and myoepithelial components), or mixed (featuring epithelial and/or myoepithelial cells with areas of cartilage or bone formation) (Pena et al., 2014).

Due to shared features in spontaneous development, clinical behavior, histopathology, and prognosis, dogs with CMTs are considered excellent comparative models for human breast cancer research (Hussain et al., 2018; Kumar et al., 2019). The canine mammary gland exhibits a complex branching architecture and diverse immunohistochemical profiles (Sorenmo et al., 2013). The heterogeneous histological and immunohistochemical nature of CMTs often complicates diagnosis (Kumar et al., 2019).

Several immunohistochemical markers, including cytokeratin, calponin, smooth muscle actin (SMA), p63, protein 14-3-3 σ , and CD-10 antigen, have been investigated to assist in identifying myoepithelial cells and improving diagnostic accuracy (Sánchez-Céspedes et al., 2013; Lopuszyński et al., 2019).

Ovarian hormones play a critical role in the etiology of mammary tumours in both species. Oestrogen receptors (ORs) and progesterone receptors (PRs) have been detected in benign and malignant CMTs (Chang et al., 2009). Notably, intact, young female dogs in estrus are more likely to develop receptor-positive tumors than older, spayed, or anestrus dogs (Sorenmo et al., 2013).

The pathogenesis of CMTs involves complex interactions among genetic mutations, epigenetic changes, hormonal dynamics, and alterations in the tumour microenvironment (Carvalho et al., 2013). Epithelial-mesenchymal transition (EMT), a key process wherein epithelial cells lose their adhesion and gain invasive mesenchymal characteristics, is central to tumour progression and metastasis (Dongre and Weinberg 2019).

This study correlated the expression patterns of E-cadherin (E-CAD), N-cadherin (N-CAD), Ki-67, vimentin and hormonal receptors in CMTs to enhance diagnostic precision and support informed treatment decisions, including surgery and adjuvant therapy. While previous research has assessed individual biomarkers in CMTs, few have taken an integrated approach. This study aimed to fill that gap by carrying out a comprehensive molecular profile to better predict tumor progression and therapeutic response in dogs.

MATERIALS AND METHODS

Tissue samples collection

Twenty-two canine mammary tumour (CMT) samples were obtained via excision biopsy (mastectomy or nodulectomy) from female dogs (n = 22) submitted to the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta. These cases originated from various private veterinary clinics and the University's Veterinary Teaching Hospital between 2013 and 2023.

Histopathological examination

Tissues were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. Sections measuring 4-5 µm were cut from each block for haematoxylin and eosin (H&E) staining. Histological evaluations were independently performed by four pathologists. Additional sections were prepared for immunohistochemistry (IHC) analysis. Clinical data—including breed, age, body size, tumour location, ovariohysterectomy (OHE) status, and lymph node involvement or metastasis (based on cytological evaluation) were extracted from accompanying case history forms. Tumours were histologically classified and graded according to the modified World Health Organization system (Goldschmidt et al., 2011) into grades I, II, and III. Staging followed the Tumour–Node–Metastasis (TNM) system, depending on the tumour size: T₁ (<3 cm), T₂ (3–5 cm), and T₃ (>5 cm) (Sorensen et al., 2003).

Immunohistochemistry (IHC)

Immunohistochemical analysis was done using a panel of antibodies targeting E-cadherin, N-cadherin, Ki-67, vimentin, progesterone receptor (PR), and oestrogen receptors (OR α and OR β). The 2-Step plus Poly-HRP anti-rabbit IgG detection system was used to detect antigen expression in neoplastic mammary tissues. Sections were dewaxed, rehydrated, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. Antigens were retrieved by heating the sections in 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven (650 W) for 10 minutes, which was followed by cooling for 20 minutes. Non-specific binding was blocked using normal goat serum for 30 minutes. Sections were incubated overnight at 4 °C with primary monoclonal antibodies against E-cadherin (Bioss, USA), N-cadherin (Elabscience, USA), Ki-67 (Novus, USA), vimentin (Novus, USA), and anti-progesterone receptor (Abcam, UK), as well as polyclonal antibodies against OR α and OR β (Abcam, UK). Secondary antibody incubation (polyperoxidase–anti-rabbit IgG) was done at room temperature for 30 minutes. Immunoreactivity was visualized using 3, 3'-diaminobenzidine (DAB), counterstained with haematoxylin, dehydrated, and mounted. Normal canine mammary gland and uterine tissues served as positive controls (Table 1).

Table 1: Primary antibodies used in immunohistochemistry

Antibody	Type/Clone	Dilution	Source	Positive control tissue
E-Cadherin	Rabbit/Polyclonal	1: 400	Bioss, USA	Mammary gland
N-Cadherin	Rabbit/Polyclonal E-AB-70061	1: 400	Elabscience, USA	Mammary gland
Ki-67	Rabbit/Polyclonal NB500-170	1: 150	Novus, USA	Mammary gland
Anti-Progesterone	Mouse/Monoclonal PR-AT4.14	1: 100	Abcam, U.K	Mammary gland
Anti-Oestrogen α	Rabbit/Polyclonal Ab 37438	1: 100	Abcam, U.K	Uterus, Mammary
Anti-Oestrogen β	Rabbit/Polyclonal Ab 3577	1: 100	Abcam, U.K	Uterus, Mammary

Source: Research's compilation (2025)

Immunohistochemical analysis

Out of the 22 canine mammary tumor samples collected, only 9 consisted of sufficient, well-preserved tissue available for the IHC analyses of E-cadherin, N-cadherin, and Ki-67. The remaining samples were depleted during diagnostic processing. In contrast, all 22 samples were suitable for the molecular analysis

of OR α . Following the immunohistochemical (IHC) procedure, each slide was examined under a light microscope for general assessment. Quantitative image analysis was subsequently performed to evaluate the expression of E-cadherin, N-cadherin, vimentin, Ki-67, OR α , OR β , and PR in tumour epithelial cells using AmScope pathology imaging software (AmScope Technologies, Chester Hill, NSW, Australia). Oestrogen receptors (OR α and OR β) were scored as described by Benavente et al. (2019). At 400 $\times$ magnification, 10 representative high-power fields per slide were selected randomly from representative tumour areas, avoiding necrotic regions and tissue edges. The proportion score (PS) was calculated as the percentage of positively stained cytoplasm or nuclei (brown signal) relative to the total area (positive + haematoxylin-stained). PS was rated on a scale of 0–5: 0 (no staining), 1 (<1%), 2 (1–10%), 3 (10–33%), 4 (33–66%), and 5 (66–100%). Intensity score (IS) evaluated staining intensity on a 0–3 scale: 0 (none), 1 (faint), 2 (moderate), 3 (strong). The total score (TS = PS + IS) categorized expression as negative (0–2) or positive (3–8) (Allred et al., 1998).

For PR and vimentin, the immunoreactive score (IRS) method was applied (Gartner et al., 1999; Arihiro et al., 2010). The percentage of positive cells was graded as: 0 (none), 1 (<10%), 2 (10–50%), 3 (51–80%), and 4 (>80%), with intensity scored as 0 (none), 1+ (weak), 2+ (moderate), and 3+ (strong). Final IRS ranged from 0 to 12: 0–1 (negative), 2–3 (mild), 4–8 (moderate), and 9–12 (strong). E-cadherin and N-cadherin expression was assessed semi-quantitatively based on the percentage of positive cells: <10%, 10–50%, or >50%. Ki-67 expression was determined by counting 50–100 tumour cells and calculating the labeling index (LI) as: Ki-67 LI (%) = (Number of positive nuclei / Total nuclei counted) $\times$ 100. Proliferative activity was categorized as low (<15–20%), moderate (20–40%), or high (>40%) (Markov et al., 2024). These cut-offs were adopted provisionally due to the lack of standardized thresholds for canine mammary tumours.

Statistical analysis

The results were analysed using descriptive statistics for each of the variables: tumour classification, histological subtypes, tumour size, location, staging, histological grade and lymph node metastasis. Means were compared in ANOVA; the Chi-square test was used to analyse the total score of the Allred TS and IRS for the associations between the expression of different antigens between benign and malignant CMTs. All statistical analyses were done with SPSS version 27 software. The p-values <0.05 were considered significant.

RESULTS

A total of 10 dog breeds were represented in the study, with the German shepherd (45.5%), and Lhasa apso (13.6%) being the most frequent. The mean

age of the affected was 8.15 years (range 4-13 years). No dog had undergone ovariohysterectomy. Most tumours were stage T₁ (n=8, 36.4%), and T₂ and T₃ were equally represented (31.8% each). T₂ and T₃ stages were more frequent in malignant tumours (77.3%) and T₁ in benign growths (22.7%). CMTs were mostly located on the left or right inguinal mammary gland, 40.9% in each; 18.2% (n=4) cases (malignant tumours) had lymph node involvement (Table 2).

Table 2: Prevalence, clinical, and pathological characteristics of CMTs

Variables	N (%)
Breed	
Pure breed	9 (90)
German shepherd	10 (45.5)
Lhasa apso	3 (13.6)
Cane Corso	1 (4.5)
Terriers	1 (4.5)
Samoyed	1 (4.5)
Dobermann	1 (4.5)
Rhodesian ridgeback	1 (4.5)
Rottweiler	1 (4.5)
Local	1 (4.5)
Mixed breed	1 (10)
Mongrel	2 (9.1)
Ovariohysterectomy	
Intact	22 (100)
Spayed	0
Tumour size	
T ₁ (<3cm)	7 (31.8)
T ₂ (3-5cm)	8 (36.4)
T ₃ (>5cm)	7 (31.8)
Tumour location	
Cranial thoracic mammary glands	2 (9.1)
Caudal abdominal mammary glands	2 (9.1)
Inguinal mammary glands	18 (81.8)
Lymph node involvement	
Yes	4 (18.2)
No	18 (81.8)

Histopathology

In the study, a total of 9 histological subtypes of CMT were diagnosed, out of which 22.7% (n=5) were benign and 77.3% (n=17) malignant. CMTs were diagnosed at stages T₁ (36.4%, n=8), T₂ (45.5%, n=10), and T₃ (18.2%, n=4). Stages T₁, T₂, and T₃ occurred more frequently in malignant tumours (77.3%) ($P = 0.03$) and all benign tumours were stage T₁ (22.7%). Regarding malignancy, grade I and grade II were observed: grade II was more frequent (72.7%, n=16) in comparison to grade I, which was 4.5% (n=1) (Table 3 and Figures 1A-F).

Table 3. Association between histological subtype with tumour staging and histological malignancy grading

Histological subtype	N (%)	Staging (%)	Histological grading (%)
Malignant	17 (77.3)		
Malignant myoepithelioma	2 (9.1)	V (9.1)	II (9.1)
Carcinoma (tubular)	4 (18.2)	I (4.5) II (9.1) V (4.5)	II (18.2)
Ductal carcinoma	1 (4.5)	II (4.5)	II (4.5)
Squamous cell carcinoma - Spindle cell variant	1 (4.5)	V (4.5)	II (4.5)
Inflammatory carcinoma	4 (18.2)	I (4.5) II (13.6)	II (18.2)
Carcinoma (solid type)	3 (13.6)	I (4.5) II (9.1)	II (9.1) I (4.5)
Carcinoma (mixed type)	2 (9.1)	II (9.1)	II (9.1)
Benign	5 (22.7)		
Simple adenoma	3 (13.6)	I (13.6)	0
Complex adenoma	2 (9.1)	I (9.1)	0
		<i>p</i> value	<i>p</i> value
		0.027	0.028

Chi-square ($P < 0.05$ was considered significant)

IHC expression of the receptor and cell differentiation

E-cadherin, N-cadherin, and vimentin were expressed in both the nuclei and cytoplasm of neoplastic epithelial cells, while ORa expression was observed in the cytoplasm of neoplastic epithelial cells; PR and Ki-67 expressions were confined to the nuclei of neoplastic cells. The negative controls were characterized by the absence of immunostaining (Table 4).

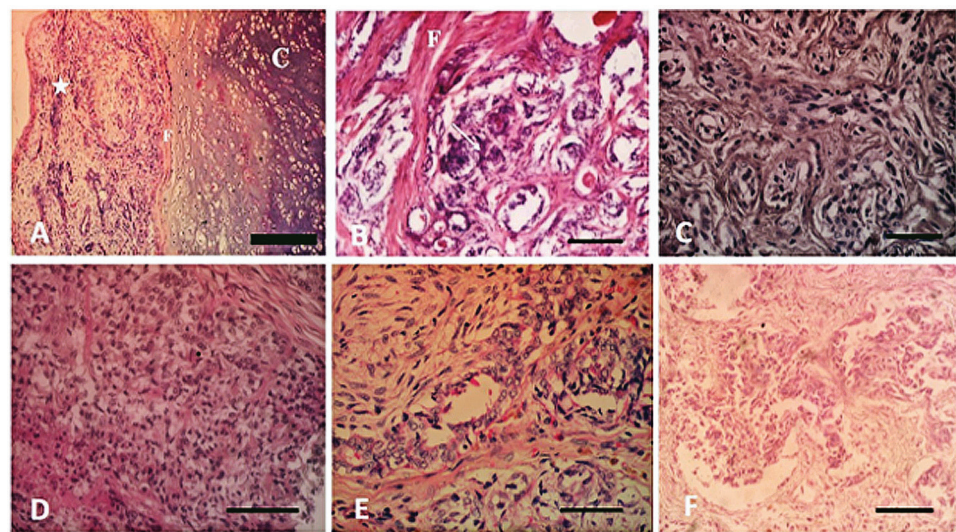


Figure 1. A. Mammary carcinoma (mixed type), neoplastic myoepithelial cells (star) and a large area of cartilage formation with an area of calcification (C) separated by fibrous connective tissue (F), H&E Stain. Scale bar=100µm; B. Mammary carcinoma, hyperchromatic myoepithelial cells (arrow) and separated by fibrous connective tissue (F); C. Carcinoma (tubular type), hyperchromatic myoepithelial cells and presence of mitotic figure; D. Simple carcinoma (solid type), neoplastic cells in solid sheets and cords; E. Complex adenoma, epithelial (tubular) and myoepithelial proliferation with spindle cells with poorly demarcated cell borders and a moderate amount of cytoplasm; F. Inflammatory carcinoma, mononuclear cells infiltration of the acini. H&E Stain. Scale bar=20µm.

Table 4. Immunohistochemical expression of E-cadherin, N-cadherin, Ki-67, Vimentin, OR α , OR β , and PR in canine mammary tumours

Immunohistochemical marker	Malignant CMT	Benign CMT	<i>p</i> value
	Positive Staining n (%)	Positive Staining n (%)	
E-cadherin	8(89)	1(11)	0.003
N-cadherin	9 (100)	0 (0)	0.001
Ki-67	5 (56)	4(44)	0.002
Vimentin	4 (18.2)	0 (0)	0.487
OR α	17 (77.3)	5 (22.7)	0.009
OR β	0 (0)	0 (0)	-
PR	9 (40.9)	0 (0)	0.214

Chi-square ($P < 0.05$ was considered significant)

E-cadherin expression

Out of the 9 samples analysed for E-CAD, 8 (89%) were immunopositive: both malignant and benign tumours expressed the antigen. The expression was predominantly observed in mesenchymal cells irrespective of the tumour malignancy (Figure 2A). However, the expression was mild in benign tumours, while malignant tumours showed severe to moderate expression. The mean E-CAD TS was 1.41 ± 0.150 for the malignant tumours and 1.00 ± 0.00 for benign tumours. The association between the E-CAD TS and the classes of tumours was not significant ($p=0.097$, Figure 3).

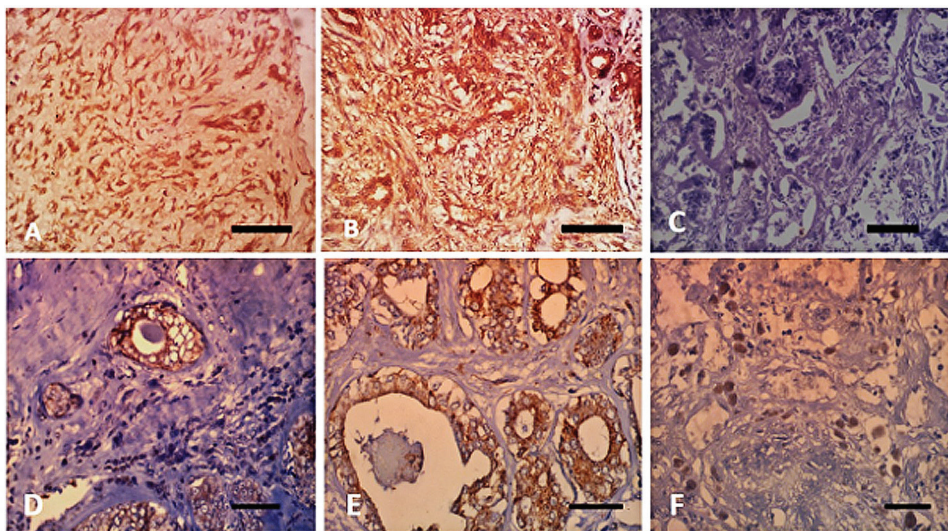


Figure 2. A. E-cadherin, IHC, diffuse severe cytoplasmic expression, mesenchymal cells (carcinoma, mixed type), scale bar=20µm; B. N-cadherin, IHC, diffuse severe cytoplasmic expression, epithelial cells, (mammary carcinoma, tubular), scale bar=20µm; C. Ki-67, IHC, mild nuclear expression, epithelial cells (inflammatory carcinoma), scale bar=20µm; D. Vimentin, IHC, moderate nuclear expression, mesenchymal cells (carcinoma, mixed type), scale bar=20µm; E. ORα, IHC, severe cytoplasmic expression by epithelial cells (mammary carcinoma, complex), scale bar=20µm; F. Progesterone, IHC, moderate nuclear expression, epithelial cells (carcinoma), scale bar=20µm.

N-cadherin expression

All of the 9 samples analysed for N-CAD were immunopositive, both the malignant and benign tumours expressed the antigen. The cells that mostly expressed the N-cadherin antigen were the epithelial cells of both the benign and malignant tumours (Figure 2B). The expression was severe in malignant tumours, and mild to moderate in the benign tumours. The mean N-CAD TS was 1.88 ± 0.81 for the malignant tumours and 1.00 ± 0.00 for benign tumours ($p = 0.001$). There

was a significant association between the N-CAD TS and the classes of tumours (Figure 3).

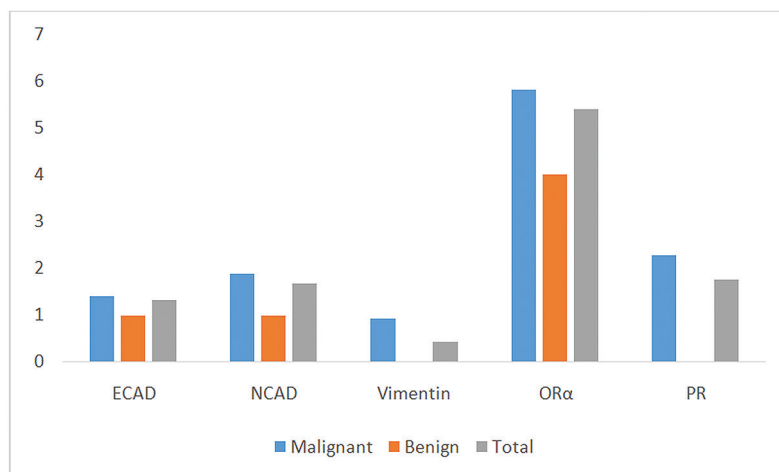


Figure 3. Mean immunohistochemical expression of E-cadherin, N-cadherin, vimentin, ORα, and PR in benign and malignant tumours. Malignant tumours generally showed higher expression levels compared with benign tumours, with statistically significant increases observed for N-cadherin, vimentin, ORα, and PR.

Ki-67 expression

Out of 9 samples analysed for Ki-67, very few were immunopositive; both the malignant and benign tumours expressed the antigen in a very mild degree. The epithelial cells of both the benign and malignant tumours mostly expressed Ki-67 antigen in this study. The expressions were mild in malignant tumours and benign tumours (Figure 2C).

Vimentin expression

About 18% ($n = 4$) of cases expressed immunopositive reactions for vimentin, in both the nuclei and cytoplasm of mesenchymal cells (Figure 2D). Half of them were moderately positive and the rest mildly positive. Vimentin expression was absent in benign tumours. The mean vimentin IRS for the malignant tumours was 0.94 ± 0.441 ($p = 0.269$, Figure 3).

Oestrogen receptor expression

Twenty-two samples were analysed for ORα, and all proved immunopositive (Figure 2E). The mean ORα TS in malignant tumours (simple and mixed) was 5.82

± 1.13 and 4.00 ± 0.00 in benign tumours (simple and complex) ($p = 0.002$, Figure 3). There was no expression for OR β .

Progesterone receptor expression

Out of 22 samples analysed for PR, 9 (40.9%) were immunopositive; all were of malignant tumour origin. Progesterone receptor expression was present mainly in the epithelial cells (Figure 2F): 31.8% were moderately positive and 9.1% mildly positive. The mean PR IRS was 2.29 ± 0.561 for the malignant tumours ($p = 0.041$, Figure 3). See also Supplementary table 1.

DISCUSSION

Mammary gland tumours have been shown over the years to be hormonally regulated in both dogs and humans, resulting from similar hormone dependency between human breast cancer and CMT (Sleeckx et al., 2011). Several studies have demonstrated the use of naturally occurring mammary gland tumours and other tumour types in dogs as sentinels for comparative studies between different types of mammary gland tumours and other tumour types in humans for diagnosis, prognosis, and development of novel therapies (Ranieri et al., 2013; Goebel and Merner, 2017). In this study, purebred dogs were overrepresented, which agrees with the findings of Vascellari et al., (2016): they reported a higher prevalence of CMT in purebred dogs, and a general increase in CMT, similar to that of human breast tumours. Only intact female dogs were affected in the current research, which affirmed the link between hormonal exposure (especially oestrogen and progesterone) and mammary tumour development. Moreover, unlike in some other countries, dog spaying at a young age is not a common practice in Nigeria, which may additionally influence the rise in the incidence of CMT. Similarly, the studies by Sorenmo et al. (2013) and Brønden et al. (2010) also reported that tumour incidence depends on the study region and the characteristics of the targeted population. The mean age of the affected dogs in this study aligns with the literature data (Sleeckx et al., 2011).

Stages T₂ and T₃ were more frequent in malignant tumours in this study, which is consistent with the results by Cassali et al. (2014), who reported that larger tumours are significantly associated with higher malignancy potential and poorer prognosis. However, it has been reported that the tumour size is irrelevant if the local lymph node is involved (Kurzman and Gilbertson, 1986). In this study, a few cases of malignant CMT had lymph node involvement, suggesting the risk of metastasis, a key prognostic indicator, which is comparable to previous studies noting metastasis rates between 10–50% depending on histological grade (Pérez Alenza et al., 1997). The inguinal mammary glands were the most common sites (40.9%), which aligns with previous findings that the caudal mammary glands are more frequently affected

due to their larger volume and higher hormonal responsiveness (Karayannopoulou et al., 2005).

Among malignant tumours, grade II was the most common, but benign tumours were only of grade I, confirming that higher grades are more indicative of malignant transformation. The high occurrence of histological grade II tumours also reflects the data provided by Pena et al. (2014), who reported it as the most prevalent in malignant CMTs, often associated with moderate differentiation and intermediate prognosis.

Simple carcinoma was significantly more frequent in this study, in which simple carcinoma (tubular) and inflammatory carcinoma occurred most. This contrasts with Vascellari et al. (2016), who reported a significantly higher incidence of complex carcinoma in their study. This could result from the difference in the study region, available samples, and the unwillingness of some dog owners to pay for diagnostics.

Both benign and malignant tumours expressed E-cadherin, although the latter showed stronger expression, contrary to many studies, which claimed that the loss of E-cadherin is associated with tumour invasiveness and metastasis (Gama et al. 2007). E-cadherin is typically considered an epithelial marker, and its expression in mesenchymal-like cells is unexpected. In this study, the mesenchymal cells were detected based on morphology in the tissue sections, but some of these cells may have retained epithelial characteristics or represented areas of tumour cells undergoing partial epithelial–mesenchymal transition (EMT). Therefore, the observed E-cadherin expression likely reflects heterogeneity within the tumour microenvironment rather than true mesenchymal cell expression. However, comparably to our findings, some studies have reported variable expression patterns of E-cadherin depending on tumour subtype and cell differentiation (Łopuszyński et al., 2015). The borderline association suggests a complex role of E-cadherin in tumour biology, beyond a simple on-off mechanism. This research found a statistically significant increase in N-cadherin expression in malignant tumours, supporting the role of epithelial–mesenchymal transition (EMT) in tumour progression. These results are consistent with Yoshida et al. (2014), who reported that increased N-cadherin expression correlates with aggressive phenotypes in canine mammary tumours (CMTs), and support the “cadherin switch” hypothesis as a hallmark of epithelial–mesenchymal transition (EMT), within the limitations of the sample size analysed.

Vimentin was expressed only in malignant tumours, further supporting its role as a mesenchymal marker in EMT processes. Although not statistically significant, its presence in a subset of malignant cases reflects the findings by Leonel et al. (2017), who showed that vimentin co-expression with epithelial markers may be indicative of invasiveness and stem-like tumour behaviour.

Only mild expression of Ki-67 was observed, with no significant difference between tumour classes. This contrasts with prior studies, where higher Ki-67 index

was significantly associated with malignant and poorly differentiated tumours (Salaritabar et al., 2019). The discrepancy in this study could be attributed to sample size limitations or variations in fixation and antigen retrieval protocols. Also, the observed low proliferative activity may suggest the predominance of low-grade tumours and the heterogeneity of canine mammary tumours, which can display lower Ki-67 indices compared to more aggressive tumours.

The strong expression of OR α in both tumour types supports the evidence that hormone receptor status is not always inversely correlated with malignancy (Nieto et al., 2000). This finding also aligns with Millanta et al. (2005), who emphasized the heterogeneity of OR α expression in CMTs and suggested that it could influence tumour behaviour and response to hormonal therapy.

The selective expression of PR supports the idea that PR may be a marker of hormonal responsiveness in malignant CMTs. Several studies have similarly found that PR expression may be retained even in more aggressive tumour forms (Chang et al., 2005), and that PR-negative tumours are often associated with poor prognosis.

CONCLUSION

This study supports that the known CMT patterns in age, intact status, tumour size, and location are critical factors. The observed associations of N-cadherin, OR α , and PR with tumor characteristics are preliminary and require validation in larger-scale studies before these markers can be considered reliable biomarkers of malignancy. The findings in this study have confirmed the similarities between CMT and human breast cancer, and suggested CMT as a spontaneous model for human breast cancer. The heterogeneous groups of different molecular-driven tumours were identified in this study; we recommend that comparative research on homogeneous groups of CMT should be carried out for more reliability and that oestrogen subtypes should be considered during therapy administration. To the best of the authors' knowledge, there has not been any documented study on the immunohistochemical study of CMT in this region.

Authors' contribution

TMO: Conceptualization, investigation, designing, data analysis, supervision, and writing of the original draft. OLA: Conceptualization, designing, supervision and writing – review. TMO & OLA: Writing – review. TMO, OLA, FMM & JTO: Sample collection and writing. The first draft of the manuscript was written by TMO and all authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical statement

No ethical approval was obtained because this study did not involve laboratory animals and consisted solely of non-invasive procedures (collection of tissue after surgery). All tissues were residual clinical specimens collected during routine surgery, and no additional procedures were performed for research purposes. This retrospective study was performed on archived diagnostic samples. All data were anonymized, and no owner consent was required according to institutional guidelines.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors state that there are no competing interests to declare.

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KLINIČKOPATOLOŠKA I IMUNOHISTOHEMIJSKA PROCENA TUMORA MLEČNE ŽLEZDE KOD INTAKTNIH KUJA

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Kratak sadržaj

Tumori mlečne žlezde kod pasa (TMŽP) spadaju u najčešće dijagnostikovane tumore kod intaktnih pasa ženskog pola. Po biološkim i hormonskih osobinama slični su tumorima mlečne žlezde kod žena, zbog čega predstavljaju dobar model u komparativnoj onkologiji. Ovaj rad ima za cilj da evaluiira kliničke, patohistološke i imunohistološke odlike TMŽP kod intaktnih ženki pasa. Ukupno su ispitivana 22 arhivirana slučaja TMŽP na Saveznom poljoprivrednom univerzitetu u Abeokuti, iz perioda od 2013. do 2023. godine. Uzorci tkiva dobijeni su od kuja mastektomijom ili nodulektomijom, koje su bile dovedene na privatne veterinarske klinike ili u nastavnu bolnicu. Beleženi su klinički podaci, uključujući starost životinja, veličinu tumora i njihovu lokaciju, prisustvo u limfnim čvorovima, i reproduktivni status kuja. Većina pasa bila je rase nemački ovčar (45,5%) prosečne starosti 8,15 godina; nije bilo sterilisanih.

Većina tumora (77,3%) bila je maligna i obično lokalizovana u ingvinalnim limfnim čvorovima (40,9%). Stadijum T₂ (45,5%) i histološki gradus II (72,7%) bili su najzastupljeniji. E-kaderin je bio imunopozitivan kod 89% tumora, a njegova ekspresija veća kod malignih. Ekspresija N-kaderina bila je značajno povezana sa malignitetom tumora ($p = 0,001$), što ukazuje na epitelno-mezenhimnu tranziciju (EMT). Estrogenski receptori α (OR α) su bili veoma izraženi kod svih tumora, i bili su na značajno višim nivoima kod malignih ($p = 0,002$). Progesteronski receptori (PR) i vimentin bili su detektovani samo kod malignih tumora, dok je Ki-67 ispoljio blagu, neznačajnu ekspresiju. Ovi nalazi sugerišu da N-kaderin, OR α i PR mogu biti potencijalni biomarkeri maligniteta i hormonske responzivnosti kod TMŽP. Oni takođe podržavaju relevantnost molekularnog profilisanja u dijagnostici i rešavanju pitanja tumora mlečne žlezde kod kuja.

Ključne reči: Tumori mlečne žlezde pasa, komparativna onkologija, imunohistohemija, N-kadherin, estrogenski receptori