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ORIGINAL ARTICLE

Epidemiological and clinicopathological characteristics of mammary tumors in female dogs: A retrospective analysis

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ABSTRACT. Mammary tumors are common neoplasms in female dogs and pose a clinical challenge because of their high malignancy potential. This retrospective study analyzed the medical records of 724 female dogs with 1,638 mammary tumors treated between 2012 and 2022 to assess epidemiological and clinicopathological associations. Malignant tumors (84.6%) were larger than benign ones (3.4 cm vs. 2.3 cm, *P*<0.01) and were associated with advanced age, multiple tumors, size >5 cm, and ulceration. Tumors >5 cm in size were associated with histological grade III, ulceration, adhesion, and regional metastases. In the multivariate analysis, tumor malignancy was 3.2 times more likely with each additional tumor, 3.3 times more likely with each additional year of age, and 4.8 times more likely with ulcerated tumors. These findings highlight the importance of early diagnosis, comprehensive evaluation, and regular monitoring, especially in high-risk dogs such as elderly and overweight female dogs.

Keywords: dog; epidemiology; mammary gland; mammary neoplasia; prognosis

INTRODUCTION

Mammary tumors (MT) account for up to 62.7% of tumors in female dogs (Rodríguez *et al*., 2022), with more than 50% of malignant tumors (Zheng *et al*., 2022; Vascellari *et al*., 2016; Salas *et al*., 2015; Ariyarathna *et al*., 2022; Rasotto *et al*., 2017; Dolka *et al*., 2024). These tumors are often associated with unfavorable prognosis (Silva *et al*., 2023; Dolka *et al*., 2024). The growing concern among dog owners regarding their pets' well-being (Withrow *et al*., 2013) combined with the search for more effective and specific treatments for each type of neoplasia highlights the importance of studies on the epidemiology and pathogenesis of cancer. Additionally, updated epidemiological studies are crucial to understand disease dynamics and identify risk factors (Salas *et al*., 2015).

Various factors influence the incidence, pathogenesis, and progression of MT in female dogs, including advanced age and obesity (Burrai *et al*., 2020; Santos *et al*., 2020; Banchi *et al*., 2021; Oliveira *et al*., 2022; Silva *et al*., 2023). The clinical and pathological aspects of tumors, such as location, size, ulceration, adherence, subtype, and histological grade, directly influence the diagnostic and therapeutic approaches for patients (Cassali *et al*., 2020). Additionally, some of these factors have been considered interrelated in some studies, such as advanced age and malignant behavior (Nunes *et al*., 2018; Burrai *et al*., 2020; Gedon *et al*., 2020; Santos *et al*., 2020), overweight and regional metastases, overweight and high histological grade (Lim *et al*., 2015), tumor size, ulceration (Soares *et al*., 2023), and tumor size and regional metastases (Gedon *et al*., 2020). Studying the relationship between these characteristics helps establish more precise prognoses.

Although clinical and pathological characteristics, such as tumor size, histological grade, number of tumors, and regional and distant metastases, are well-studied individually, few studies have examined the relationship between these tumor-specific characteristics and dog-specific factors, such as age and body condition score (BCS) (Rao *et al*., 2009; Burrai *et al*., 2020; Banchi *et al*., 2021). This gap in the literature limits our understanding of how these factors are interconnected, which could provide a basis for developing preventive strategies for dog owners.

The objective of this study was to perform an epidemiological analysis of MT in female dogs treated at the Veterinary Hospital of the Federal University of Uberlandia (HOVET-UFU) from 2012 to 2022, and to evaluate the association between epidemiological and clinicopathological characteristics (e.g., age, BCS, number of neoplastic nodules, tumor behavior, histological grade, tumor size, and regional and distant metastases) to support prevention and prognostic criteria.

MATERIALS AND METHODS

The medical records of female dogs diagnosed with MT, spayed or not, who were voluntarily referred for treatment at HOVET-UFU between January 2012 and December

2022, were retrospectively evaluated. Each medical record contained an authorization form for future studies signed by the owners or guardians of the animal.

Female dogs that underwent clinical evaluation, chest radiography, abdominal cavity ultrasound, mastectomy, and histopathological diagnosis of MT (benign or malignant) were included in the study. The exclusion criteria were as follows: female dogs that did not undergo mastectomy, those that lacked thoracic radiography and abdominal ultrasonography results, and those that had a histopathological diagnosis indicating non-neoplastic lesions. The collected data included age, breed, BCS, tumor size, presence of ulceration and adherence, histological subtype and grade, presence of regional metastases (with histopathological diagnosis), and radiographic or ultrasonographic findings suggestive of metastases in distant organs. The animals were grouped as puppies (<1 year), adults (1- 8 years), and elderly (>8 years) (Goldston & Hoskins, 1999). The BCS was determined using a 1 to 9 scale (1 to 3, very thin; 4 or 5, ideal weight; 6 or 7, overweight; 8 and 9, obese) (Laflamme, 1997). For statistical analyses, the female dogs were divided into two groups: ideal BCS (very thin and ideal) and overweight (overweight to obese).

Lesions and regional lymph nodes were histopathologically evaluated by examining MT samples on hematoxylin and eosin-stained slides from tissues fixed in 10% buffered formalin. The sample processing was performed as described by Tolosa *et al*. (2003). The diagnosis was conducted in a double-blind study by two trained pathologists, using the classification by Goldschmidt *et al*. (2011) as a reference. Mammary tumors were classified as benign or malignant and histologically graded as I, II, or III, according to the Nottingham system modified by Elston and Ellis (1991). Female dogs with malignant tumors were staged using the tumor-node-metastasis (TNM) staging system. Inflammatory carcinoma was diagnosed based on clinical and histopathological correlations as outlined by Goldschmidt *et al*. (2011). The diagnosis was made after careful consideration of the clinical presentation and histological features of the tumors.

The presence or absence of an association between the following variables was verified using the chi-square test: number of tumors (tumor behavior, age), age (tumor behavior, histological grade, regional metastases, staging), BCS (histological grade, regional metastases, staging, and tumor behavior), tumor size (regional metastases, ultrasonographic changes suggestive of distant metastases, histological grade, ulceration, adhesion, and tumor behavior), ulceration (tumor behavior), and adhesion (tumor behavior). The Chi-square test residuals were evaluated in contingency tables with more than two rows or columns to identify the associated variables. For the variables associated with malignancy that were significant in the Chi-Square Test, multiple logistic regression was performed to assess the simultaneous impact of different factors on malignant diagnosis. The model included age, number of tumors, ulceration, and tumor size. The analysis was conducted on dogs with complete data for all evaluated variables. The significance level was set at $P<0.05$. Student's t-test for independent samples was used to compare the age of female dogs with benign and malignant tumors and the size of benign and malignant tumors. The Wilcoxon test was used to compare the ages of overweight and ideal-weight female dogs because of the non-normality of the data. Data analysis was performed using R software (v 3.2.2). A significance level of 5% was considered for all statistical analyses. Only female dogs with complete information regarding the evaluated variables were included in each analysis.

RESULTS

The study included 716 female dogs (Table 1) and 1638 mammary tumors (MT) (Table 2). Among the identified MT, 84.6% were classified as malignant and 15.6% as benign (Table 2). Single tumors were observed in 321 dogs (44.3%) and 403 dogs (55.7%) had multiple tumors (Table 3). The average number of MT per dog was 2.3 (SD = 1.9). Their ages ranged from one to 20 years (mean = 9.5; SD = 3.1). No dogs younger than 1 year of age were affected; therefore, for statistical analyses, the dogs were regrouped into the adult and senior categories. The average tumor size was 3.3 cm (SD = 3.6), ranging from 0.1 cm to 25 cm. Malignant tumors had a larger average size (mean = 3.4 cm) than benign tumors (mean = 2.3 cm, $P<0.01$).

According to histological subtypes, 9.3% (67/724) of the dogs presented only benign tumors, 9% (6/67) had different histological subtypes (Table 2), and 91% (61/67) had the same histological subtype. Additionally, 75.4% (546/724) of the dogs had only malignant tumors, with 36.7% (266/546) showing different histological subtypes and 71.6% (391/546) showing the same subtype, while 15.3% (111/724) had both benign and malignant tumors simultaneously. Ultrasound findings suggestive of distant metastases were detected in 17% (115/724) of the animals, with 11.3% (13/115) showing changes in more than one organ simultaneously. The organs affected included the spleen (47.9%, 57/119), lungs (26.9%, 32/119), liver (14.3%, 17/119), uterus (3.4%, 4/119), urinary bladder (1.7%, 2/119), intestine (1.7%, 2/119), lymph nodes (1.7%, 2/119), ovaries (0.8%, 1/119), subcutaneous tissue (0.8%, 1/119), and vagina (0.8%, 1/119). Regional lymph nodes were histopathologically evaluated in 311 dogs diagnosed with malignancies, and 33.4% (104/311) showed metastasis. Dogs with multiple tumors showed a higher frequency of malignant MT (57.8%, 380/657) than those with a single MT (42.2%, 277/657) ($P<0.01$).

A significant association was observed between the number of tumors and patient age ($P<0.01$; Table 1). Among the 714 dogs analyzed, 61.9% (270/436) of senior dogs and 46.4% (129/278) of adult dogs had multiple MT. Additionally, age was associated with tumor behavior ($P<0.01$), with 62.7% (406/648) of malignant diagnoses occurring in senior dogs and 37.3% (242/648) in adult dogs. Dogs with malignant

Table 1. Clinical and epidemiological profile of female dogs with benign and malignant mammary tumors treated from 2012 to 2022 at the Veterinary Hospital of the Federal University of Uberlândia-MG.

Variable	Total	Benign	Malignant
Purebred or mixed-breed			
Purebred	377 (52.7%)	37 (56.9%)	340 (52.2%)
Mixed-breed	339 (47.3%)	28 (43.1%)	311 (47.8%)
Total	**716**	**65**	**651**
Breed			
Poodle	100 (26.5%)	3 (8.1%)	97 (28.5%)
Pinscher	47 (12.5%)	5 (13.5%)	42 (12.4%)
Shih tzu	34 (9%)	4 (10.8%)	30 (8.8%)
Pitbull	30 (8%)	3 (8.1%)	27 (7.9%)
Basset hound	28 (7.4%)	5 (13.5%)	23 (6.8%)
English Cocker Spaniel	18 (4.8%)	2 (5.4%)	16 (4.7%)
Yorkshire	18 (4.8%)	2 (5.4%)	16 (4.7%)
Dachshund	12 (3.2%)	0 (0%)	12 (3.5%)
Labrador	12 (3.2%)	2 (5.4%)	10 (2.9%)
Boxer	11 (2.9%)	2 (5.4%)	9 (2.7%)
Maltese	10 (2.7%)	1 (2.7%)	9 (2.7%)
Rottweiler	9 (2.4%)	0 (0%)	9 (2.7%)
Lhasa apso	8 (2.1%)	0 (0%)	8 (2.4%)
Brazilian Mastiff	7 (1.9%)	0 (0%)	7 (2.1%)
Dalmatian	5 (1.3%)	2 (5.4%)	3 (0.9%)
German Shepherd	3 (0.8%)	1 (2.7%)	2 (0.6%)
Fox terrier	3 (0.8%)	0 (0%)	3 (0.9%)
Beagle	3 (0.8%)	1 (2.7%)	2 (0.6%)
Chow chow	2 (0.5%)	1 (2.7%)	1 (0.3%)
Schnauzer	2 (0.5%)	0 (0%)	2 (0.6%)
Blue heeler	2 (0.5%)	0 (0%)	2 (0.6%)
Border collie	2 (0.5%)	0 (0%)	2 (0.6%)
Golden retriever	2 (0.5%)	1 (2.7%)	1 (0.3%)
Bulldog	2 (0.5%)	1 (2.7%)	1 (0.3%)
Akita	2 (0.5%)	0 (0%)	2 (0.6%)
Dobermann	1 (0.3%)	1 (2.7%)	0 (0%)
Bull terrier	1 (0.3%)	0 (0%)	1 (0.3%)
Bichon frise	1 (0.3%)	0 (0%)	1 (0. 3%)
Swiss Shepherd	1 (0.3%)	0 (0%)	1 (0.3%)
Belgian Shepherd	1 (0.3%)	0 (0%)	1 (0.3%)
Total	**377**	**37**	**340**
Age			
Adult	278 (38.9%)	36 (54.5%)	242 (37.3%)**
Senior	436 (61.1%)	30 (45.5%)	406 (62.7%)
Total	**714**	**66**	**648**

(Cont)

Variable	Total	Benign	Malignant
Body Condition Score			
Very Thin	17 (2.8%)	0 (0%)	17 (3.1%)*
Ideal weight	162 (27%)	12 (22.2%)	150 (27.5%)
Overweight	286 (47.7%)	31 (57.4%)	255 (46.7%)
Obese	135 (22.5%)	11 (20.4%)	124 (22.7%)
Total	**600**	**54**	**546**
TNM staging system			
I	86 (25.4%)	-	86 (25.4%)
II	62 (18.3%)	-	62 (18.3%)
III	44 (13%)	-	44 (13%)
IV	90 (26.6%)	-	90 (26.6%)
V	56 (16.6%)	-	56 (16.6%)
Total	**338**	-	**338**

* P=0.05; ** P=0.01

Table 2. Histological subtypes of benign and malignant mammary tumors in female dogs treated from 2012 to 2022 at the Federal University of Uberlândia-MG.

Histological subtype		Frequency	Lesions (%)
Benign			
Benign mixed tumor	91	36%	5.60%
Complex adenoma	63	24.90%	3.90%
Simple adenoma	55	21.70%	3.40%
Fibroadenoma	15	5.90%	0.90%
Tubular adenoma	14	5.50%	0.90%
Myoepithelioma	12	4.70%	0.70%
Tubulopapillary adenoma	2	0.80%	0.10%
Papillary adenoma	1	0.40%	0.10%
Total	**253**	**100%**	**15.60%**
Malignant			
Epithelial origin			
Mixed tumor carcinoma	368	28.70%	22.50%
Complex carcinoma	275	21.40%	16.80%
Tubular carcinoma	232	18.10%	14.20%
Tubulopapillary carcinoma	154	12%	9.40%
Solid carcinoma	92	7.20%	5.60%
Carcinoma in situ	42	3.30%	2.60%
Papillary carcinoma	36	2.80%	2.20%
Squamous cell carcinoma	31	2.40%	1.90%
Micropapillary carcinoma	16	1.30%	1%

(Cont) Histological subtype		Frequency	Lesions (%)
Anaplastic carcinoma	15	1.20%	0.90%
Ductal carcinoma	7	0.60%	0.40%
Adenocarcinoma	6	0.50%	0.40%
Mucinous carcinoma	4	0.30%	0.20%
Inflammatory carcinoma*	3	0.20%	0.20%
Fusiform carcinoma	1	0.10%	0.10%
Pleomorphic lobular carcinoma	1	0.10%	0.10%
Invasive lobular carcinoma	1	0.10%	0.10%
Total	**1.284**	**100%**	**78.60%**
Mesenchymal origin			
Hemangiosarcoma	37	43.50%	2.30%
Osteosarcoma	15	17.70%	0.90%
Malignant myoepithelioma	12	14.10%	0.70%
Fibrosarcoma	10	11.80%	0.60%
Mixed tumor sarcoma	4	4.70%	0.20%
Chondrosarcoma	4	4.70%	0.20%
Liposarcoma	3	3.50%	0.20%
Total	**85**	**100%**	**5.15%**
Epithelial and mesenchymal origin			
Carcinosarcoma	16	100%	1%
Grand total	1638	100%	100%

* Note: The inflammatory carcinoma subtype was confirmed through histopathological analysis in conjunction with clinical evaluation of the animal.

Table 3.
Number of mammary tumors per female dog attended at the Veterinary Hospital of the Federal University of Uberlândia-MG from 2012 to 2022.

N° of tumors	N° of dogs (%)
1	321 (44.3%)
2	160 (22.1%)
3	103 (14.2%)
4	71 (9.8%)
5 or more tumors	69 (9.5%)

Table 4.
Clinical-pathological characteristics of benign and malignant mammary tumors in female dogs attended at the Veterinary Hospital of the Federal University of Uberlândia-MG from 2012 to 2022.

Variable	N	Benign	Malignant
		Size	
T1	839 (59.1%)	151 (70.2%)	688 (57.1%)**
T2	337 (23.7%)	46 (21.4%)	291 (24.1%)
T3	244 (17.2%)	18 (8.4%)	226 (18.8%)
Total	**1420**	**215**	**1205**
		Ulceration	
Ulcerated	190 (16.7%)	10 (5.7%)	180 (18.7%)**
Non-ulcerated	949 (83.3%)	166 (94.3%)	783 (81.3%)
Total	**1139**	**176**	**963**
		Adhesion	
Adherent	140 (13.5%)	18 (11.1%)	122 (14%)*

tumors had a higher average age (mean = 9.7 years, SD = 3.1) than those with benign tumors (mean = 8.2 years, SD = 3) (*P*<0.01; Table 1). No association was found between age and the presence of regional metastases (*P*>0.05), histological grade (*P*>0.05), or staging (*P*>0.05).

To evaluate the association between BCS and histological grade, 335 dogs were included in this analysis. Dogs with ideal weight had a higher frequency of Grade I MT (59.1%, 68/115) and a lower frequency of Grade II MT (32.2%, 37/115) than overweight dogs (Grade I: 45.9%, 101/220; Grade II: 45.5%, 100/220) (*P*=0.05). The frequency of Grade III MT was similar between the groups. Grade I was associated with ideal weight and Grade II was associated with overweight status. No association was found between BCS and tumor behavior (*P*>0.05), staging (*P*>0.05), or regional metastases (*P*>0.05).

Regarding the association between tumor size and behavior, the sample included 839 tumors < 3 cm (T1), of which 82% (688/839) were malignant (Table 4). Tumors between 3 and 5 cm (T2) were 337, with 86.4% (291/337) being malignant, whereas tumors > 5 cm (T3) totaled 244, with 92.6% (226/244) being malignant. Tumor size was significantly associated with tumor behavior (*P*<0.01), with benign behavior associated with T1, and malignant behavior with T3. To determine the association between tumor size and regional metastases, 361 dogs were included. Regional metastases were observed in 21.9% (35/160) of dogs with T1 tumors, 30.9% (34/110) of dogs with T2 tumors, and 38.5% (35/91) of dogs with T3 tumors, with T3 being associated with the presence of regional metastases (*P*<0.05). A total of 827 tumors were evaluated for size and histological grade. Of the 513 T1 tumors, 55.4% were Grade I, 38.4% were Grade II, and 6.2% were Grade III. Among the 189 T2 tumors, 51.3% were Grade I, 42.9% were Grade II, and 5.8% were Grade III. Of the 125 T3 tumors, 38.4% were Grade I, 50.4% were Grade II, and 11.2% were Grade III. Grade I tumors were associated with tumors < 3 cm, whereas Grade III tumors were associated with tumors > 5 cm (*P*<0.01). Tumor size was also associated with ulceration, which included 1, 073 MT. Among the T1 tumors, 6% (37/622) were ulcerated, 23.2% (60/259) of T2 tumors were ulcerated, and 39.1% (75/192) of T3 tumors were ulcerated. T1 tumors were associated with the absence of ulceration, whereas T2 and T3 tumors were associated with ulceration (*P* < 0.01). In the analysis of the association between tumor size and adhesion, 975 tumors were included. The frequencies of adhered MT were: T1 (7.5% - 42/563), T2 (17.4% - 41/236), and T3 (27.3% - 48/176). T1 tumors were associated with the absence of adhesion, whereas T2 and T3 tumors were associated with adhesion (*P*< 0.01).

A significant association was found between tumor behavior and ulceration, with 94.7% (180/963) of ulcerated tumors being malignant and 5.3% (10/176) benign (*P*<0.01). Malignant tumors were 3.8 times more likely to ulcerate (OR = 3.8). However, no association was found between adhesion and tumor behavior (*P*>0.05).

Multiple logistic regression was performed on 523 dogs to assess the simultaneous impact of age, number of tumors, ulceration, and tumor size on malignant diagnosis. The results indicated that dogs with ≥ 2 tumors had a higher risk of malignancy than those with a single tumor (OR = 3.2, 95% CI: 1.2–8.4, *P*<0.02). Similarly, older dogs (≥ 8 years) had higher odds of malignancy than adults (< 8 years) (OR = 3.3, 95% CI: 1.3–8.3, *P*<0.01). Ulceration was also significantly associated with malignancy (OR = 4.8, 95% CI: 2.0–11.5, *P*<0.04). In contrast, tumor size (T2: 3–5 cm and T3: >5 cm, compared with T1: ≤3 cm) (T2: OR = 1.6, 95% CI: 0.5–5.2, *P*=0.4; T3: OR = 1.3, 95% CI: 0.8–4.0, *P*=0.6) showed no significant impact on malignancy (Table 5).

Table 5.
Multivariate Logistic Regression for Malignant Tumor Diagnosis in Female Dogs Attended at the Veterinary Hospital of the Federal University of Uberlândia-MG from 2012 to 2022.

Variable	Comparison Group	Reference group	Odds Ratio (IC 95%)	*P*-value	Association with Malignancy
N° of Tumors	≥2 tumors	1 tumor	3.2 (1.2 – 8.4)	0.02	Higher number of tumors - Higher malignancy risk
Age	Senior (≥8 years)	Younger dogs	3.3 (1.3 – 8.3)	0.01	Older dogs - Higher malignancy risk
Ulceration	Yes	No ulceration	4.8 (2.0 – 11.5)	0.04	Presence of ulceration - Higher malignancy risk
Tumor Size	T2 (3–5 cm)	T1 (≤3 cm)	1.6 (0.5 – 5.2)	0.4	No significant impact
	T3 (>5 cm)	T1 (≤3 cm)	1.3 (0.8 – 4)	0.6	No significant impact

DISCUSSION

Malignant and multiple tumors were more frequent, as indicated in previous studies (Sorenmo *et al*., 2009; Dias *et al*., 2016). The high frequency of malignant MT may vary depending on the location of the study. For example, a study conducted in Portugal reported that 53.1% of canine TMs are benign (Carvalho *et al*., 2023). This discrepancy might be related to the habits of the study population. In our study, it was observed that many dog owners wait for the tumor to grow or multiply before seeking veterinary care. This behavior should be discouraged, as demonstrated in this study, where larger and multiple TMs, which are likely associated with a longer growth period, indicate a worse prognosis. Multivariate logistic regression analysis showed that for each additional tumor, the likelihood of malignancy increased by 3.2 times. Furthermore, the possibility of benign tumors progressing to malignant tumors over time has been reported (Sorenmo *et al*., 2009). Early diagnosis allows the treatment of TMs at an early stage when they are still small and solitary, enabling more effective and less invasive therapies (Pecile *et al*., 2021).

The average number of nodules per animal was 2.3, with different histological types coexisting in female dogs with multiple MT. Histological diagnosis, which is crucial for determining the prognosis, can only be performed after mastectomy. Some subtypes have a life expectancy of only three months, necessitating the use of adjuvant therapies (Rasotto *et al*., 2017). In this study, a single female dog exhibited multiple histological types. Therefore, it is essential that all tumors undergo histopathological diagnosis, not just the largest one, because they may not represent the histological type with the worst prognosis.

Malignant tumors were larger than benign tumors, as observed in other studies (Burrai *et al*., 2020; Ariyarathna *et al*., 2022; Zheng *et al*., 2022). Despite the association between T3 size and malignant tumors in the univariate analysis, in the multivariate analysis, the effect of size may have been attenuated owing to its strong correlation with characteristics such as ulceration. It is possible that ulceration absorbed the effect of size; however, this interpretation should be considered with caution as other factors not evaluated may have influenced the results. T3 tumors were associated with the presence of regional metastases, histological grade III, ulceration, and adhesion, indicating greater invasive and growth potential. These observations highlight the clinical relevance of tumor size as an indicator of negative prognosis. In fact, it has been reported that T3 tumors are associated with shorter survival times (Rasotto *et al*., 2017; Oliveira *et al*., 2022). They also exhibit higher cell proliferation rates and lower expression of hormonal receptors (Ferreira *et al*., 2009), which may explain the association found in this study between larger tumor size and worse prognosis.

In this study, a high frequency of ulcerations and adhesions was observed. Ulcerated tumors were predominantly malignant and were 4.8 times more likely to be malignant, while adhesion was not associated with malignant behavior. Ulceration can be a strong indicator of malignancy; however, its absence does not guarantee benign diagnosis. Ulceration results from increased tumor diameter (Nunes *et al*., 2018; Soares *et al*., 2023) and reflects a high rate of cell proliferation, leading to skin rupture. Consistently, in the present study, larger tumors were associated with a higher frequency of ulceration. In addition to indicating aggressiveness, ulceration is undesirable as it causes pain, discomfort, and an increased risk of infection.

On imaging exams, 17% of the animals showed changes suggestive of distant metastasis. Although the lungs are generally the most affected organs (Cassali *et al*., 2020), in this study, the spleen was the most affected organ. The frequency of distant metastasis may have been underestimated because all female dogs had undergone mastectomy (Cassali *et al*., 2020). Female dogs with pulmonary metastases were probably more frequently excluded from surgical procedures owing to anesthesia limitations. The frequency of regional metastases was 33.4%, which is consistent with previous reports (Rasotto *et al*., 2017; Tesi *et al*., 2020). This high frequency underscores the importance of histopathological examination of the lymph nodes in female dogs with MT (Goldston & Hoskins, 1999), as the presence of regional metastasis negatively impacts survival (Rasotto *et al*., 2017; Nunes *et al*., 2018).

Regarding the histopathological diagnosis, the group of epithelial-origin tumors was predominant, while the groups of mesenchymal-origin and epithelial-mesenchymal (mixed) tumors, considered rare compared to others, were also a minority in this study (Nunes *et al*., 2018; Tesi *et al*., 2020). The most common histological subtypes were carcinoma in mixed tumors, complex carcinoma, tubular carcinoma, and tubulopapillary carcinoma, which were predominantly malignant. Other studies have also reported that these histological subtypes are more frequent (Rao *et al*., 2009; Rodríguez *et al*., 2022) and are associated with better prognosis (Tolosa *et al*., 2003; Rasotto *et al*., 2017). Among the benign tumors, benign mixed tumors, complex adenomas, and simple adenomas stood out, with findings similar to those of other studies (Tesi *et al*., 2020; Rodríguez *et al*., 2022; Prates *et al*., 2023).

Carvalho *et al*. (2023) reported a low occurrence of inflammatory carcinoma in both female dogs (0.6%) and women (0.1%), a type of carcinoma that is aggressive, has a poor prognosis, and is highly metastatic (Marconato *et al*., 2009). In our study, the observed frequency was even lower (0.2%), which may not accurately reflect the true prevalence. This is likely due to the fact that all dogs in the study underwent mastectomy, a surgical procedure that is not recommended for cases of inflammatory carcinoma, given the lack of response to this treatment and the characteristic clinical and histopathological behavior of the disease (Cassali *et al*., 2020).

Older female dogs were more affected, and their mean age was similar to those reported in other studies (Daleck *et al*., 1998; Tesi *et al*., 2020; Oliveira *et al*., 2022; Soares *et al*., 2023). Additionally, female dogs with exclusively malignant tumors had a higher mean age, consistent with other studies (Sorenmo *et al*., 2009; Nunes *et al*., 2018; Burrai *et al*., 2020; Banchi *et al*., 2021; Carvalho *et al*., 2023). Tumor behavior was associated with age, with 62.7% of tumors in older female dogs being malignant. Older female dogs showed an increased risk of developing malignant tumors, similar to that reported by other authors (Jing *et al*., 2024). The risk of tumors increases by 1.6 times (Santos *et al*., 2020) for each additional year of life. Furthermore, in this study, for each additional year of age, the likelihood of malignancy increased by 3.3 times. Many elderly female dogs face limitations in anesthetic and surgical procedures, and the presence of multiple tumors may further complicate surgical planning. Given these observations, regular monitoring of older dogs with periodic examinations for early tumor detection is essential. Although older dogs were expected to show a higher histological grade, advanced staging, and a higher frequency of regional metastases due to the generally unfavorable prognostic indices associated with age, this was not observed in this study. A possible explanation could be that in cases of advanced tumors and metastatic spread, some older dogs were not candidates for mastectomy. Therefore, they were excluded from this study.

Overweight dogs were predominant in the present study. Being overweight has been identified as a risk factor associated with a 2.3-fold increase in tumor risk (Santos *et al*., 2020), which was associated with histological grade II. Although the frequency of grade III tumors did not differ between the two groups, the association between overweight and grade II tumors corroborates other studies, where grades II and III were associated with overweight (Tesi *et al*., 2020; Prates *et al*., 2023). One factor that may have contributed to the lack of association between BCS and grade III histology is a limitation of this study, in which the number of tumors classified as grade III was small. As overweight dogs with MT typically have shorter survival (Tesi *et al*., 2020), it was expected that they would be associated with other poor prognostic characteristics. However, there was no association between BCC and regional metastases, staging, or tumor behavior.

Purebred dogs were more frequent (Table 1), differing from some studies (Daleck *et al*., 1998; Oliveira *et al*., 2022) and corroborating others (Salas *et al*., 2015; Nunes *et al*., 2018; Ariyarathna *et al*., 2022; Zheng *et al*., 2022; Dolka *et al*., 2024). Studies reporting a higher frequency of tumors in mixed-breed dogs attributed this result to the characteristics of the studied population (Daleck *et al*., 1998; Oliveira *et al*., 2022). Although mixed-breed dogs were predominant at the hospital where this study was conducted (Lim *et al*., 2015), purebred dogs still prevailed. This information may indicate a genetic predisposition to tumors in purebred female dogs. However, breed identification by dog owners may have been inaccurate, with mixed-breed dogs mistakenly identified as purebred. While some authors argue that there is no breed predisposition to MT (Daleck *et al*., 1998), others suggest that because of the lower genetic diversity within each breed, certain breeds may accumulate risk alleles over time, making them more susceptible (Silva *et al*., 2023). For example, a study involving five generations of female beagles identified two distinct maternal lineages with different phenotypes, one susceptible and the other resistant to MT (Schafer *et al*., 1998). There is no consensus on which breeds are most predisposed to MT, as the results vary depending on the study's location, time period (Schafer *et al*., 1998), design, and potential biases (Vazquez *et al*., 2023). In this study, poodle was the most affected breed, which is consistent with previous reports (Salas *et al*., 2015; Tesi *et al*., 2021; Oliveira *et al*., 2022; Silva *et al*., 2023; Soares *et al*., 2023; Dolka *et al*., 2024), indicating an increased risk of MT in this breed (Jing *et al*., 2024; Silva *et al*., 2023).

The limitations of this study include inaccuracies in the information provided by dog owners, such as the breed. Additionally, owing to the retrospective nature of the study, some data were missing from patients' medical records.

CONCLUSION

This study highlights the predominance of malignant and multiple MT in female dogs, emphasizing the importance of early diagnosis and comprehensive histopathological evaluation of all nodules. Ulceration and advanced age were associated with malignancy, reinforcing their clinical relevance as prognostic indicators. These findings underscore the need for regular monitoring and preventive measures, particularly for high-risk female dogs, such as the elderly and overweight individuals, to improve outcomes and effectively guide treatment strategies.

Competing Interests Statement

The authors declare that they have no competing interests.

Ethics Statement

The study was approved by the Ethics Committee on Animal Use of the Federal University of Uberlandia under protocol 104/2022.

Data availability statement

The authors declare that all data supporting the findings of this study are available within the article. Additional data can be made available upon request to the corresponding author.

Author Contributions

SSM: Data collection, data analysis, interpretation of results, manuscript writing, and final approval of the manuscript. TCM: Data collection, interpretation of results, manuscript revision, and final approval of the manuscript. VMFM: Data collection, interpretation of results, manuscript revision, and final approval of the manuscript. AAMR: Conceptualization, study design, data collection, data analysis, interpretation of results, manuscript revision, supervision, and final approval of the manuscript.

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