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Research Article

Determination of hypoxia, angiogenesis and tumour microenvironment in feline mammary tumours by immunohistochemical and histopathological methods

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ABSTRACT

This study used immunohistochemical method to investigate the relationship between the tumor microenvironment, hypoxia, and angiogenesis in biopsy samples of feline mammary tumors brought to Selcuk University Faculty of Veterinary Medicine and Bornova Veterinary Control Institute between 2015 and 2019. The staining of paraffin tissue sections was performed with CD31, vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1 (HIF-1a) CD68, and CD163 antibodies, and their correlation with each other and the observed histopathological changes was explored. The study used mature mammary tissue samples from 12 cats of different breeds and ages for diagnostic purposes. The examined biopsy materials with microvessel density (MVD), VEGF, HIF-1a, CD68, and CD163 antibodies showed no significant relationship between benign and malignant tumors ($p>0.05$). Furthermore, the study found no significant relationship between malignant tumors and their histological grade, tumor size, mitotic score, lenfovascular invasion (LVI), and necrosis features ($p>0.05$). It is believed that the low number of materials used in the study prevented the detection of a statistically significant difference between the IHC results of tumors and their histopathological and clinicopathological features. The study concluded that presenting the data would be appropriate to contribute to the fields of veterinary medicine and veterinary oncology.

INTRODUCTION

Mammary tumors rank third in cats, following hematopoietic and skin tumors (Misdorp, 2002). Goldschmidt et al. (2017) have reported that the annual incidence rate in cats is 25.4/100,000. When examined by age, the frequency of occurrence increases in cats over nine years old, with the highest diagnosis rates between 10 and 12 years of age. Despite the incomplete understanding of the breed predisposition for mammary tumors in cats, reports indicate a higher incidence in the Siamese breed (Goldschmidt et al., 2017).

The tumor microenvironment defines the cells and structures surrounding the cancer cells in the tumor, including the tumor's vascular system, lymphatics, fibroblasts, pericytes, and sometimes adipocytes (Balkwill et al., 2012). Various non-tumor cells in the tumor microenvironment also affect the behavior of tumor cells (Sun et al., 2014).

Macrophages make up a significant portion of the infiltrated leukocytes in the tumor tissue (Murdoch et al., 2004). Tumor-associated macrophages (TAMs) classify these cells as either anti-tumoral (M1) or pro-tumoral (M2) macrophages, depending on their activation status in response to microenvironmental changes (Chanmee et al., 2014; Mantovani and Locati, 2013). In the tumor microenvironment, TAMs are very important. They affect the movement of tumor cells, the breakdown and remodeling of the extracellular matrix, the growth of new blood vessels, and the metastasis and invasion of tumor cells (Chanmee et al., 2014; Zhang et al., 2012).

Hypoxia is the most important metabolic change in the tumor microenvironment, and 50–60% of solid tumors have heterogeneously distributed hypoxic and anoxic areas within the tumor mass (Vaupel, 2004; Vaupel and Mayer, 2007). To adapt to hypoxic conditions, cells undergo a series of changes in gene expression and function. Many alterations in gene expression during hypoxia are caused by the activation of hypoxia-inducible factor (HIF) (McNeil et al., 2016). HIF allows tumor cells to adapt to and survive in hypoxic conditions in the tumor microenvironment by reprogramming genes involved in angiogenesis, glycolytic metabolism, oxygen consumption, invasion, and migration (Rapisarda and Melillo, 2009).

The formation of new blood vessels is termed angiogenesis. Tumors require new blood vessels to grow beyond 1-2 mm in size. Angiogenesis facilitates the progression, metastasis, and invasion of tumor tissue, providing the oxygen, growth factors, and nutrients necessary for the tumor's development (McNamara et al., 1998).

Because of the clinicopathological, histological, and epidemiological similarities between feline and human mammary cancers, feline mammary tumors can be used as models for human mammary tumors in cancer research. In addition, studies in feline mammary tumors may provide clues to better understand the mechanisms of cellular response, angiogenesis, and hypoxia in the tumor microenvironment. In this study, it was aimed to determine the relationship of paraffin tissue sections of cat mammary tumor biopsy samples with the tu-

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mor microenvironment, hypoxia, and angiogenesis by immunohistochemical method. For this purpose, we evaluated the immunexpressions of CD31, vascular endothelial growth factor (VEGF), HIF-1a, CD68, and CD163 antibodies used in staining and investigated the relationship between them and the observed histopathological changes.

MATERIALS and METHODS

The material for the study consisted of mature mammary tissue samples from 12 cats of various breeds and ages, brought for diagnostic purposes to Selcuk University Faculty of Veterinary Medicine and Bornova Veterinary Control Institute between 2015 and 2019. After the tissue samples of the mammary tissues obtained by the surgical operation were sent to the pathology laboratories of the above-mentioned departments, they were examined macroscopically and tissue samples were taken from the necessary areas for examination.

Histopathological Examination

Mammary biopsy samples were fixed in 10% buffered formaldehyde solution and embedded in paraffin after routine tissue processing procedures. The tissue samples were sectioned at a thickness of 5 microns on a microtome (Leica RM 2125RT), stained with Haemotoxylene&Eosin (H&E) and examined under a binocular light microscope (Olympus BX51).

The malignancy degree of cat tumors was determined according to the newly reported numerical grading system (Goldschmidt et al. 2017).These grading systems are shown in Table 1. An Olympus BX51 microscope (with a 0.55 mm diameter field of view at x400 magnification) was used for histomorphological assessment.

Immunohistochemistry Staining and Evaluation

All sections from the cases were examined, and paraffin blocks representing the tumor most accurately were selected. The evaluation of hypoxia, angiogenesis, and tumor-associated macrophages in the tumor microenvironment was conducted using CD68, CD163, CD31, VEGF, and HIF-1α antibodies. Immunohistochemistry was done on 4-micron-thick sections obtained from paraffin tissue blocks of these cases and mounted on Poly-L-lysine-coated slides were used and subjected to tissue applications of the Novolink™ Polymer Detection System RE7150-K by Leica Microsystems, following the recommended protocol of the respective company. Clone numbers, dilution rates, and incubation times of the primary antibodies used are summarized in Table 2. Negative controls for each staining were also processed using the same procedure but with TBS instead of the primary antibody. For positive controls, hemangiosarcoma tissue from a dog was used for CD31, healthy liver tissue for VEGF, and lung tissue with pneumonia from another dog for HIF-1a, CD68, and CD163. Following staining using the same procedure for all sections, they were examined under a light microscope (Olympus BX51) and evaluated semi-quantitatively (Choudhury et al., 2010. Hameed et al., 2015; Weidner et al., 1991; Monteiro et al., 2018).

The evaluation of samples labeled with VEGF and HIF-1a was performed according to the Allred scoring method (Choudhury et al., 2010; Hameed et al., 2015; Ates, 2019). This scoring system assessed staining intensity and proportion scores in two categories, similar to standard scoring systems. According to this method, staining intensity (darkness) scores were determined as 0 (no staining), 1 (weak), 2 (moderate), and 3 (intense/dark). Proportion score was determined based on the ratio of stained cells to all cells in the examined area, as follows: 0 (no staining), 1 (>0-1/100), 2 (>1/100-1/10), 3 (>1/10-1/3), 4 (>1/3-2/3), 5 (>2/3-1). The staining intensity score and proportion score were added to determine the Allred score for each case, ranging from 0 to 8 (Hameed et al., 2015; Ates, 2019) (Table 3).

To determine microvessel density (MVD), samples were evaluated immunohistochemically using CD31 staining. Weidner et al. (1991) modified the technique for MVD determination. Initially, sections were scanned at low magnifications (x40 and x100) under a light microscope. Subsequently, areas with the highest vascularization, called 'hot spots,' were selected at x100 magnification. Microvessel counting was performed in five different 'hot spots' areas at ×200 magnification. Endothelial cells or clusters positively stained with CD31 were included in the count. Vessels in normal breast tissue, fibrosis, necrotic

Table 1. Grading of feline mammary carcinomas (Goldschmidt et al. 2017).

Histologic features		**Score**
A. Lymphovascular invasion	Absent	0
	Present	1
B. Nuclear form	< % 5 abnormal	0
	≥ % 5 abnormal	1
C. Mitotic count (10 consecutive HPF)	≤62	0
	>62	1
Total score (A+B+C)	**Grade**	
0	1	Low grade
1	2	Intermediate grade
2-3	3	High grade

(BBA: x40 magnificant; 0,55 mm diamater, Olympus BX51 microscope)

Table 2. Antibody Panel.

Antibody	Clone number	Company	Dilution Rate	Incubation Time	Cellular localization
CD31	H-3 (Mouse Monoclonal)	Santa Cruz	1:400	Room temperature/ 1 hour	Cytoplasmic
CD68	3F103 (Mouse Monoclonal)	Santa Cruz	1:50	+4°C/overnight	Cytoplasmic
CD163	GHI/61 (Mouse Monoclonal)	Santa Cruz	1:50	+4°C/overnight	Cytoplasmic
VEGF	VG-1 (Mouse Monoclonal)	Santa Cruz	1:250	Room temperature/ 1 hour	Cytoplasmic
HİF-1a	28b (Mouse Monoclonal)	Santa Cruz	1:100	+4°C/overnight	Nuclear/ Cytoplasmic

Table 3. Allred scoring method (adapted from Hameed et al (2015).

Proportion score	PS*	Intensity score	IS*
0	0	No staining	0
>0-1/100	1	Weak	1
>1/100-1/10	2	Moderate	2
>1/10-1/3	3	Intense	3
>1/3-2/3	4		
>2/3-1	5	Allred Score= PS+IS	

*PS: Proportion score, IS: Intensity score

regions, areas with inflammation, and vessels with muscle walls were excluded from the count. The average microvessel counts in the five high vascularization areas in the sections were used to calculate MVD.

Macrophages were evaluated immunohistochemically using CD68 and CD163 staining. The technique employed for macrophage counting was adapted from Monteiro et al. (2018). Initially, sections were scanned at low magnifications (x40 and x100) under a light microscope, and five areas with the highest macrophage density, referred to as 'hot spots,' were identified. Macrophage counting was performed at ×400 magnification for each area. Macrophage cells stained with CD68 and CD163 were included in the count for each area.

Statistical analysis

The statistical data analysis was conducted using the SPSS for Windows 25 software package. The Chi-square test was used for comparing groupable clinicopathological data with each other and with immunohistochemical data. The Mann-Whitney U test (for comparison between two groups) and the Kruskal-Wallis test (for comparison among more than two groups) were employed to compare mean values with other clinicopathological data. Quantitative variables were presented as mean ± standard deviation. Results were considered statistically significant at $p< 0.05$.

RESULTS

The histopathological classification of cases was conducted according to Goldschmidt et al. (2017) criteria (Table 4). Tumor types, sizes, and localization of masses, as well as information on animal species, age, and breed, were presented in Table 5 based on this classification.

Out of the 12 examined cases of mammary tumors, nine were malignant and three were benign, all of which were comprised of mastectomy materials. The ages of the cats included in the study ranged from 1 to 14 years (mean: 9.6 years). The highest incidence of mammary tumors was observed in the age range of 9–12 years (Figure 1). When evaluated by breed, the highest number of tumors was detected in the mixed breed (n = 7).

Macroscopic findings

The smallest tumor size among the cases was 2x0.8x1.2 cm, and the largest was 8x3.5x2.5 cm. The cross-sections of some cases appeared grayish-white, moist, and lobulated. In some cases, the outer parts were elastic, while the inner parts had occasional complex foci. The cut sections of these tumors were grayish-white, and areas resembling bone-like calcifications were present (Figure 2).

Histopathological Findings

The lymphovascular invasion status, tumor necrosis, mitot-

Table 4. Histologic classification of feline mammary neoplasms (Goldschmidt et al. 2017).

1. Hyperplasia/dysplasia	**3. Malignant epithelial neoplasms**	**4. Malignant epithelial neoplasms – special types**
Duct ectasia	Carcinoma – *in situ*	Squamous cell carcinoma
Lobular hyperplasia (adenosis)	Carcinoma – simple	Adenosquamous carcinoma
Regular	a. tubular	Mucinous carcinoma
With secretory activity	b. tubulopapillary	Lipid-rich (secretory) carcinoma
With fibrosis	c. cystic-papillary	Spindle cell carcinoma
With atypia	d. cribriform	Squamous cell carcinoma – spindle cell variant
Epitheliosis	Carcinoma – micropapillary invasive	Carcinoma – spindle cell variant
Papillomatosis	Carcinoma – solid	Inflammatory carcinoma
Fibroadenomatous change	Comedocarcinoma	**5. Malignant mesenchymal neoplasms – sarcomas**
Gynecomastia	Carcinoma – anaplastic	Fibrosarcoma
2. Benign neoplasms	Intraductal papillary carcinoma	Other sarcomas
Adenoma – simple	Ductal carcinoma	
Intraductal papillary adenoma		
Ductal adenoma		
Fibroadenoma		

Table 5. Mammary tumors in feline and information about animals.

No	Diagnosis	Race	Age	Localization	Size
1*	Tubular carcinoma	Mixed	14	No information	2x2,7x2,5 cm
2*	Solid carcinoma	Mixed	10	No information	3,5x1,5x1 cm
3*	Ciytic-papillary carcinoma	No information	10	No information	3x1,4x3,1 cm
4*	Solid carcinoma	Mixed	11	No information	2,5x2x1 cm
5*	Mucinous carcinoma	Mixed	11	Cranial mammary lobe	8x3,5x2,5 cm
6*	Tubular carcinoma	Siamese cat	8	No information	3,5x3,2x1 cm
7*	Tubulopapillary carcinoma	Mixed	13	No information	2x2x1,2 cm
8*	Ductal adenoma	No information	11	No information	2x0,8x1,2cm
9*	Simple adenoma	Mixed	9	No information	1,5x2x2,1cm
10*	Comedokarcinoma	Mixed	9	No information	5x3,6x1,1 cm
11**	Anaplastic carcinoma	Siamese cat	8	Inguinal mammary lobe	1,5x2x1,3 cm
12**	Simple adenoma	Persian cat	1	Cranial and caudal thoracic mammary lobe	4x1,2x3 cm

*: Bornova Veterinary Control Institute
**: Selcuk University Faculty of Veterinary Medicine

ic score, and grade information, along with histopathological diagnosis details and IHC results for the cases comprising the study material, are presented in Table 6. In two cases, corpora amylacea of varying sizes were found in ectatic glands and duct lumens (Figure 3).

Immunohistochemical Findings

CD31, HIF-1a, and VEGF antibodies labeled all cases with positive immunoreactivity. CD68 and CD163 labels revealed positive immunoreactivity in 5 and 7 cases, respectively. CD31 expression was observed rarely in endothelial cells of small and large blood vessels and occasionally in macrophages. VEGF expression was modest in endothelial cells and widespread or localized in granular form in the cytoplasm of tumor cells. Tumor cells primarily displayed HIF-1a in their cytoplasm and nucleus, particularly in perinecrotic areas. Macrophages labeled with CD68 and CD163 were observed individually or in clusters in the tumor stroma (Figure 4).

The MVD of benign tumors (40,87±18,79) in cats was lower compared to malignant tumors (49,64±15,22). The mean VEGF IHC staining score in benign tumors was 5.67±0.58, which was higher than in malignant tumors, 5.22±1.56. The mean HIF-1a IHC staining score was higher in benign tumors (5.00±1.73) compared to malignant tumors (3.44±1.42). The density of macrophages labeled with CD68 in benign tumors was 5.00±5.00, while it was 11.11±23.35 in malignant tumors. The density of macrophages labeled with CD163 in benign tumors (3.00±2.65) was higher than in malignant tumors

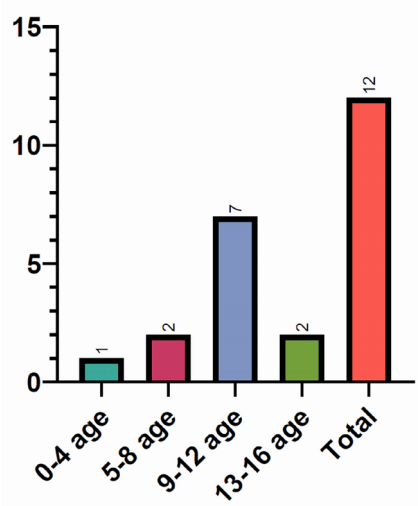

Figure 1. The distribution of mammary tumors of feline according to age.

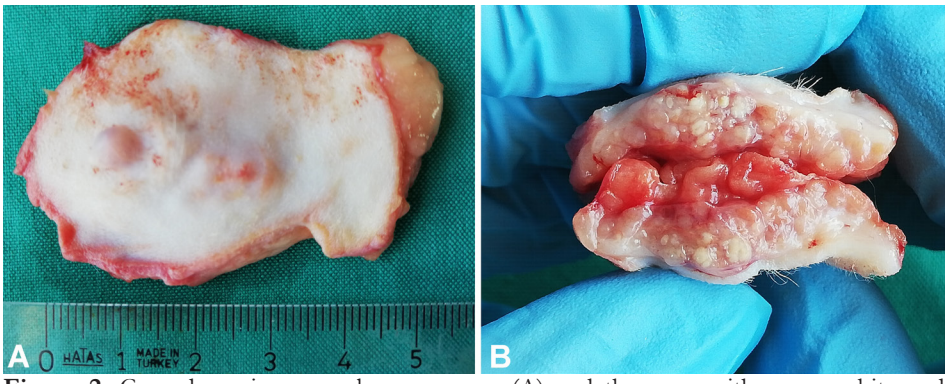

Figure 2. Comedocarcinoma, pale appearance (A) and the mass with a grey-white and lobular appearance on cut-section (B), case number:10.

Table 6. IHC scores and histopathological features of mammary tumors from cats.

No	Diagnosis	MVD (CD31)	VEGF TS	HIF-1A TS	CD68	CD163	LVI	Necrosis	Mitotic score	Grade
1	Tubular carcinoma	51	5	2	0	41	+	+	50	3
2	Solid carcinoma	37.6	6	2	29	75	+	+	82	3
3	Ciytic-papillary carcinoma	34.4	3	3	0	48	-	-	45	1
4	Solid carcinoma	28.2	8	6	0	7	-	-	25	2
5	Mucinous carcinoma	52.2	5	4	68	65	-	+	30	2
6	Tubular carcinoma	64.8	3	3	3	0	+	+	70	3
7	Tubulopapillary carcinoma	73.2	5	6	0	0	-	-	35	2
8	Ductal adenoma	55.2	5	5	0	0	-	-	-	-
9	Simple adenoma	19.6	6	4	10	5	-	-	-	-
10	Comedocarcinoma	62.8	6	6	0	0	+	+	35	3
11	Anaplastic carcinoma	42.6	6	6	0	0	+	+	85	3
12	Simple adenoma	47.8	6	2	5	4	-	-	-	-

LVI: Lenfovascular Invasion +=present, - = absent
Necrosis: + =present, - = absent
Mitotic score (BBA: x40 magnificant; 0,55 mm diamater, Olympus BX51 microscope)
TS: Total Score= Proportion Score (PS)+intensity score (IS)

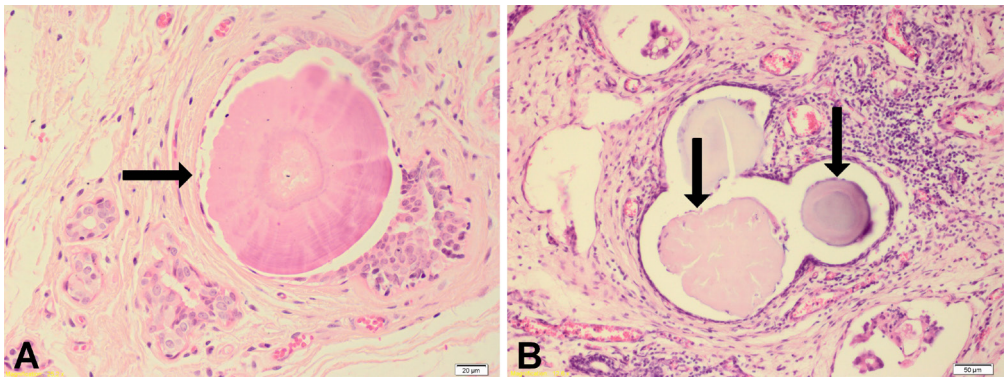

Figure 3. Corpora amylaceae of different sizes (arrows) in ectatic gland and duct lumens. A. Simple adenoma, feline (case no:9), B. Anaplastic carcinoma, feline, (case no:11).

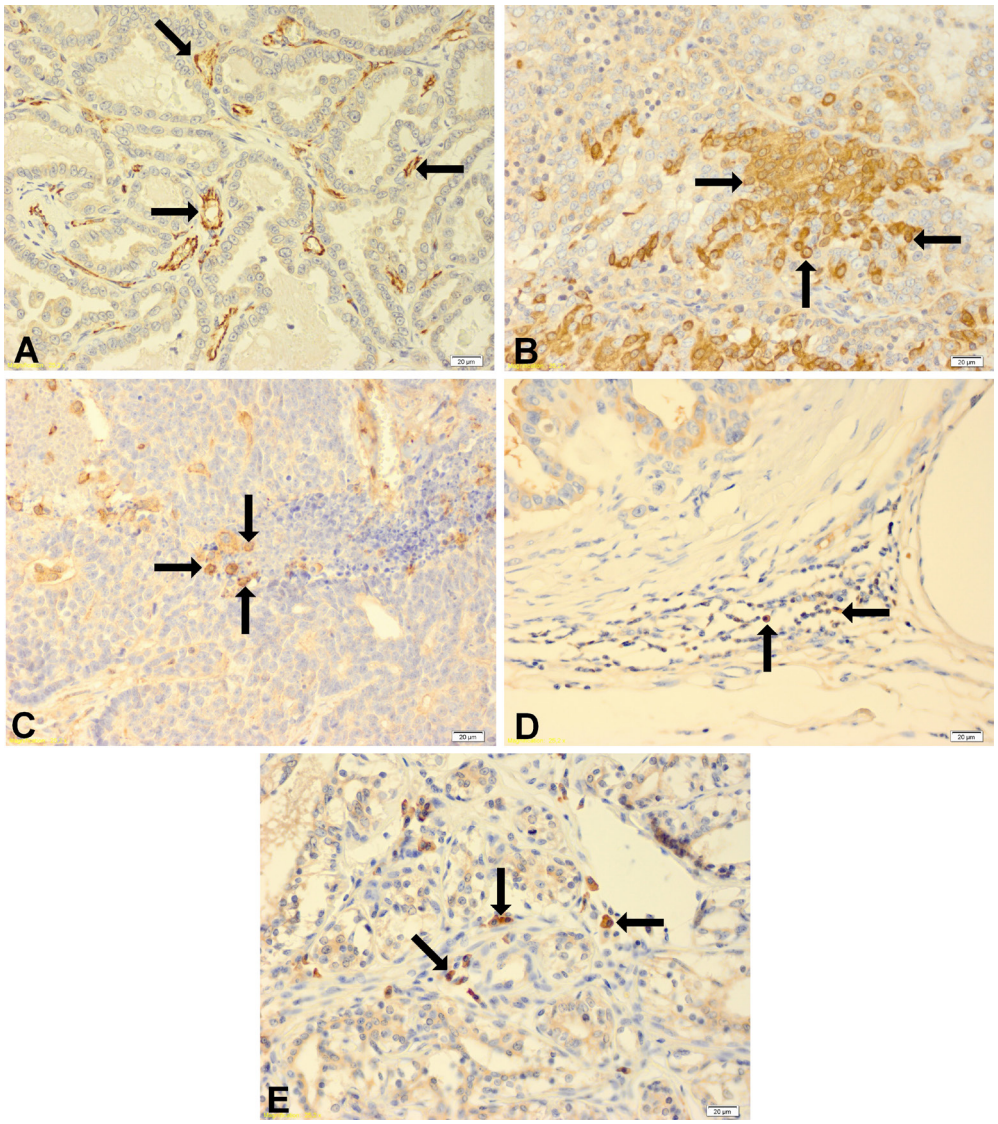

Figure 4. Tubulopapillary carcinoma, (A); Expression of CD31 in vascular endothelium among tubulopapillary structures (arrows); feline, case no:7, Bar=20 µm. Ductal adenoma, (B); Expression of VEGF in tumor cells (arrows) feline, case no:8, Bar=20 µm. Comedocarcinoma, (C);HIF-1a expression in tumor cells (arrows) around the necrotic area; feline, case no:10, Bar=20 µm. Solid carcinoma, (D); Expression of CD163 in macrophage cytoplasms (arrows);feline, case no:2, Bar=20 µm. Tubular carcinoma, (E); Expression of CD68 in macrophage cytoplasms (arrows); feline, case no:6, Bar=20 µm.

(26.22±31.02) and the difference between them was statistically insignificant in all the above-mentioned data. ($p > 0.05$) (Figure 5). In the examined malignant tumors, no statistically significant relationship was found between IHC results and histological grade, tumor size, mitotic score, LVI, and necrosis features ($p > 0.05$) (Table 7).

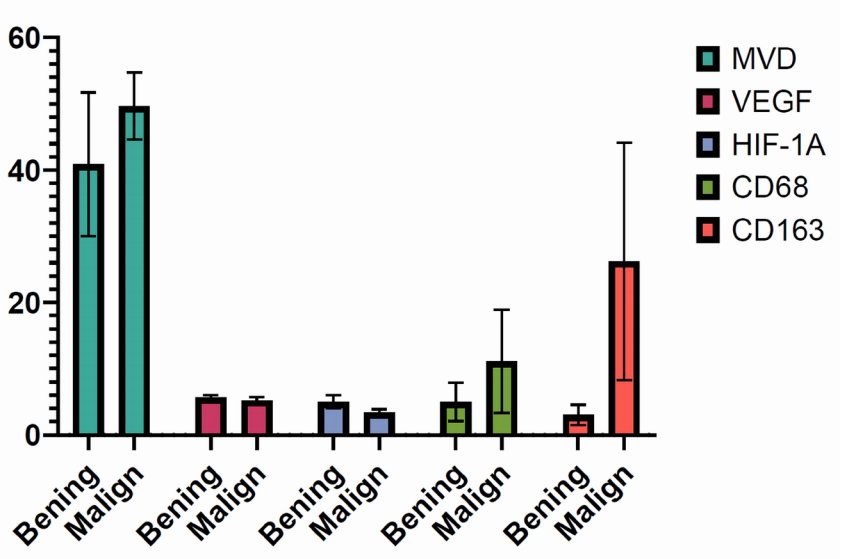

Figure 5. IHC Results in benign and malignant tumors (Mean±sd)

Table 7. Immunohistochemical results and histopathological clinicopathological features of malignant tumors in feline (Mean±sd)

		n	MVD	VEGF	HIF-1A	CD68	CD163
Histological Grade*	1	1	34.40±0.00	3.00±0.00	3.00±0.00	-	48.00±0.00
	2	3	51.20±22.52	6.00±1.73	3.00±1.00	22.67±39.26	24.00±35.68
	3	5	51.76±12.01	5.20±1.30	3.80±1.79	6.40±12.70	23.20±33.97
	p		0.509	0.364	0.820	0.864***	0.678
Tumor size *	2-3 cm	4	48.75±18.82	6.00±1.41	3.00±1.15	-	12.00±19.61
	>3cm	5	50.36±14.00	4.60±1.52	3.80±1.64	20±29.47	37.60±35.66
	p		1.000	0.413	0.556	---	0.413
Mitotic Score**	≤62	6	50.30±16.89	5.33±1.63	3.17±1.17	11.33±27.76	26.83±28.07
	>62	3	48.33±14.48	5.00±1.73	4.00±2.00	10.67±15.95	25.00±43.3
	p		1.000	1.000	0.548	0.381	0.905
LVI**	Present	5	51.76±12.01	5.20±1.30	3.80±1.79	6.40±12.70	23.20±33.97
	Absent	4	47.00±20.21	5.25±2.06	3.00±0.82	17.00±34.00	30.00±31.51
	p		0.730	0.730	0.556	0.905	0.556
Necrosis**	Present	8	46.70±13.25	5.25±1.67	3.38±1.51	12.50±24.56	29.50±31.45
	Absent	1	73.20±0.00	5.00±0.00	4.00±0.00	-	-
	p		0.222	0.889	0.667	---	---

(*) Kruskall Wallis Varyans Analysis, (**) Mann Whitney-U test, (***) Since there is no grade 1 CD68 staining, Mann Whitney-U test was performed.

DISCUSSION

This study aimed to investigate the relationship between CD31, HIF-1a, VEGF, CD68, and CD163 antibodies, which are used to investigate hypoxia, angiogenesis, and malignancy in feline mammary tumors, and their relationship with histopathological changes using immunohistochemical methods.

It has been reported that more than 85% of mammary tumors in felines are malignant (Karabolovski et al., 2020; Murphy, 2008). In the study, out of 12 mammary gland tumors diagnosed in female cats, 9 (75%) were malignant, and 3 (25%) were benign tumors.

Although the occurrence rate of mammary tumors in felines shows a noticeable increase after the age of 9, the highest diagnosis is reported to be between the ages of 10-12 (Goldschmidt et al., 2017; Hayes and Mooney, 1985). In the study, the ages of felines with mammary tumors ranged from 1 to 14 years, with an average age of 9.6 years. As for the age range, it was most commonly observed between 9 and 12 years, consistent with literature data (n=7).

Certain breeds, such as Siamese, Persian, and domestic shorthair cats, are reported to be more prone to mammary tumors (Amorim et al., 2006; Goldschmidt et al., 2017; Hayes et al., 1981; Shida et al., 2010). In the study, the highest incidence of mammary tumors in cats was observed in the mixed breed (n=7). Although this result is not very reliable due to the small number of materials evaluated, it is concluded that it cannot be definite regarding the breed characteristics in Konya and Izmir regions due to the fact that the samples of cats belonging to mixed breeds were more common in the date range in which the samples were examined.

Studies on benign and malignant mammary tumors have reported that MVDs are higher in malignant tumors than benign tumors (Jakab et al., 2008; Raposo et al., 2014; Restucci et al., 2000; Sleeckx et al., 2014). In our study, the MVD in malignant tumors in cats was higher than in benign tumors, in line with the literature data. However, the difference was statistically insignificant in both groups ($p>0.05$).

In studies, VEGF was found to be higher in malignant tumors compared to benign tumors (Qui et al., 2008; Restucci et al., 2002). Unlike these studies, in our study, VEGF was higher in benign tumors than in malignant tumors. This difference may be due to the fact that the studies were conducted on canine mammary tumors or that the number of benign mammary tumors used in our study was lower than the number of malignant tumors. Studies exploring the connection between angiogenesis and VEGF in cats are scarce. Islam et al. (2012) reported a positive correlation between VEGF expression and MVD in feline mammary tumors. Millanta et al. (2002) conducted a different study on feline mammary tumors and reported that, unlike other researchers, they did not detect a significant relationship between VEGF and angiogenesis. In our study, correlation analysis could not be performed due to the low number of materials.

Many studies have presented evidence that HIF-1a is effective in the development of aggressive tumors (Shin et al., 2015; Madej et al., 2013). The mean HIF-1a IHC score was higher in benign tumors than in malignant tumors, and the statistical difference was insignificant ($p > 0.05$). In addition, we couldn't find any relationship between clinicopathological features. Despite studies reporting a relationship between histological grade and HIF-1a, our results underscore the need for large-scale studies on this issue.

Studies reported that the number of tumor-associated macrophages was significantly higher in malignant tumors than in benign tumors. (Raposo et al., 2014; Raposo et al., 2015). Although CD68 and CD163 numbers in feline tumors were statistically insignificant, similar to the studies, they were higher in malignant tumors than benign tumors, and similar to these studies, we could not detect a significant relationship between them and features such as tumor size, histological grade, necrosis and lymph node metastasis in feline mammary tumors ($p>0.05$).

CONCLUSION

No statistically significant difference was found between the IHC results of the tumors used in the study and histopathological and clinicopathological features ($p>0.05$). This may have been due to insufficient sample numbers. Therefore, it would be more accurate to conduct multicenter studies examining histopathological and clinicopathological characteristics with more materials. The study concluded that presenting the data would be appropriate to contribute to the fields of veterinary medicine and veterinary oncology.

DECLARATIONS

Ethics Approval

For the study, Selcuk University, Faculty of Veterinary Medicine Experimental Animal Production and Research Center Ethics Committee (SUVDAMEK) approval was obtained (Date: 31.01.2019, Decision No:2019/04).

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: EG, FH

Data collection and analysis: EG

Drafting of the manuscript: EG, FH

Critical review: FH

Data Availability

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request (E. GUNER).

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This study encompasses the section on cat mammary tumors from a PhD thesis project that included dog and cat mammary tumors brought to Selçuk University Faculty of Veterinary Medicine and Bornova Veterinary Control Institute between 2015 and 2019 (Guner, 2020). The data obtained from the investigation of dog mammary tumors within the same project have been previously shared to contribute to the field of veterinary oncology (Guner and Hatipoğlu, 2023).

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