Slov Vet Res
DOI 10.26873/SVR-1800-2026

Early View

Original Research Article

Clinicopathological Evaluation of Estrogen Receptor, Progesterone Receptor, Epidermal Growth Factor Receptor and Carbohydrate Antigen 15-3 in Canine Mammary Tumour

Key words

biomarkers;
dogs;
mammary tumor;
ELISA

Çağla Nur Küçükbekir[1], Çağatay Tek[2], Zeynep Günay Uçmak[2]*, Atila Ateş[3]

[1]Institute of Graduate Education, [2]Department of Obstetrics and Gyneacology, [3]Department of Biochemistry, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye

***Corresponding author:** zeynep.gunayucmak@iuc.edu.tr

Abstract: Mammary tumors are the most common neoplasms in unspayed female dogs. Biomarkers are biological molecules measured in blood or tissue that provide information for the presence of disease, treatment and prognosis. Tumor biomarkers can be used to determine cancer risk, early diagnosis, recurrence or progression. In this study, 125 tissue samples from 31 dogs with mammary tumor were included. It is aimed to evaluate the tissue estrogen receptor (ER), progesterone receptor (PR), epidermal growth factor receptor (EGFR) and carbohydrate antigen 15-3 (CA 15-3) in regard to different tumor types and clinicopathological parameters. Tumors included in the study; were evaluated in 3 groups as malignant epithelial tumors group (MET, n=66), hyperplasia&dysplasia (H&D, n=43) group and benign mammary tumor (BMT, n=16) group. The highest ER and PR levels were determined in group BMT ($P<0.05$). Also, CA 15-3 level in group MET was significantly higher than in group H&D ($P=0.01$). However, the evaluated biomarkers in group MET were not different with regard to clinicopathological parameters such as histological subtypes and grading, tumor size, and invasion ($P>0.05$). In this study, ER was moderately and positive correlated with PR ($r=0.383$, $P<0.001$) while it was low and positive correlated with EGFR ($r=0.223$, $P<0.05$). Also, CA 15-3 was moderately and positive correlated with EGFR ($r=0.376$, $P<0.001$) while it was low and positive correlated with PR ($r=0.216$, $P<0.05$). This study stated that ELISA is capable enough to detect the ER, PR, EGFR and CA 15-3 in canine mammary tumor tissue. In conclusion, ER and PR can distinguish benign tumor from malignant tumor and H&D, while CA 15-3 can distinguish malignant tumor from H&D.

Received: 29 june 2023
Accepted: 4 March 2026

Introduction

Neoplasms are one of the most common conditions seen in dogs in recent years. While it is a leading cause of death in dogs, the Veterinary Cancer Society estimates that 25% of dogs will be diagnosed with cancer in their lifetime (1-3). Mammary tumors are the most commonly diagnosed tumors in female dogs (4). Nikodinovska et al. (5) investigated canine tumors and reported that mammary adenocarcinomas were the most frequently diagnosed tumor compared to all tumors (19.42%).

Biomarkers are biological molecules that can be measured in blood or tissues and provide information for the presence of a disease, treatment outcome and prognosis (6, 7). Tumor biomarkers can be used to determine cancer risk, early diagnosis, recurrence or progression (8).

Estrogen is thought to have an effect on tumor development (9). While estrogen receptors (ER) are found in both benign and malignant mammary tumors, ER is expressed more in benign mammary tumors (10). Progesterone receptor (PR) is one of the biomarkers that effectively predict hormonal response in mammary tumors (11). Progesterone receptor protein products from target genes are involved in cellular activities such as transcription, steroid and lipid metabolism, cell growth and apoptosis, and some of these proteins are associated with

mammary tumor development (12). The epidermal growth factor receptor (EGFR) belongs to the family of human epidermal growth factor receptors (HER) (13). It is associated with poor clinical outcomes because it increases angiogenesis and metastasis (14). The cancer antigen known as Mucin 1 (CA 15-3) is a glycoprotein that is a product of the mucin 1 gene expressed from the apical plasma membrane (15). In malignant cases, the membrane expression of the MUC-1 gene, associated with cell surface oncoproteins, often changes from apical to lateral at the same time as the loss of polarity in epithelial cells. By acting as anti-adhesive molecules, it facilitates the detachment of malignant cells. Thus, it increases the potential of tumor cells to metastasize and invade (16). High serum levels of CA 15-3 are associated with large tumor size, disease stage, and the presence of lymph node metastases, and they indicate a poor prognosis (17,18).

There are a limited number of studies in the literature evaluating the tumor tissue in dogs with mammary tumors together and investigating the relationship with clinical parameters (9, 15, 19, 20). The aims of this study are to evaluate the difference of ER, PR, EGFR, CA15-3 biomarkers in canine mammary tissue with different pathologies and to investigate the change in amounts of the specified biomarkers in malignant epithelial tumors related to clinical parameters such as histopathological subtype, mass size, invasion status, tumor grade.

Materials and methods

The animal care protocol and experimental procedures in this study were approved by the Unit Ethical Committee at the İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine (Approval Number: 2018/23).

Animal material and working procedure

Animal material was obtained from 125 mammary lobe samples from 31 dogs with mammary tumors, aged 6-14 years and weighing 6-40 kg, brought to Istanbul University-Cerrahpaşa Veterinary Faculty, Department of Obstetrics and Gynecology. Mammary tissues included in the study were examined in 3 groups as malignant epithelial tumors group (MET), hyperplasia&dysplasia (H&D) group and benign mammary tumor (BMT) group. The study groups formed according to the histopathological results of mammary tissues as bening tumors (BMT) and malignant tumors (MT). Also, Group MT divided into 2 subgroups as; malignant epithelial tumors (MET), carcinosarcomas and malignant mixed tumors. Hemogram, blood biochemistry analysis results and three view thoracic radiography of the dogs were evaluated. Distant and regional metastases were determined based on clinical examiantion, radiography, ultrasonography and histopathology. The corresponding numbers of the groups according to the clinical examination were presented in Table 1. Distant metastasis to lungs and regional lymph nodes were observed in 7 dogs. The tumors in group MET were classified according to the tumor, lymph node, metastasis (TNM) system determined by the World Health Organization (21). While the distribution of masses was present in all lobes, the distribution of MET was higher in caudo-abdominal and inguinal lobes. As a result of macroscopic and histopathological examination, ulceration was detected in 3 of the mammary tumors and inflammation in 1 of them.

Table 1: The corresponding numbers of the groups according to the clinical examination

Clinical Examination Data	Number of Samples
Groups	
MET	66
H&D	43
BMT	16
Total mass number	125
Tumor Size (T)	
T1 (0-3 cm)	37
T2 (3-5 cm)	13
T3 (> 5 cm)	16
Intramammarial Lymph Node Invasion	
(+)	18
(-)	48
Presence of Distant Metastasis	
(+)	7
(-)	24
OVH Status of the Dogs	
OVH (-)	25
OVH (+)	6

MET: Malignant epithelial tumors, H&D: Hyperplasia&Dysplasia, BMT: Benign mammary tumor, OVH: Ovariohysterectomy

Dogs were initially administered Atropine (Atropine, Vetaş, Türkiye), (0.02-0.04 mg/kg via subcutanously) as a preanesthetic. Propofol (Propofol-PF 1%, Polifarma Ilaç, Türkiye) was administered at a dose of 6-8 mg/kg for induction. General anesthesia was maintained with 3-3.5% isofluorane (Forane, Baxter, Egypt) and 2-3% oxygen. Bilateral total mastectomy was performed as a treatment.

Homogenization and analysis phase

Tissue samples were cut into small pieces, up to 500 mg each. Phosphate Buffered Saline (PBS) cooled at 4°C and 1 ml per 100 mg sample (0.01M, pH= 7.4) added. The homogenization process was carried out in ice using ultraturax (Miccra-D1). After application, homogenized samples were stored at 8°C until centrifugation. Homogenates were centrifuged using a cooled

centrifuge (Sigma 3K30) at 3000 rpm for 20 min. The supernatants were portioned into 4 pieces and stored at -25°C until analysis was performed. Samples kept at -25°C were raised to room temperature for measurements. Since four different markers will be looked at, each marker was prepared and measured separately. The MicroQuant ELISA system (Bio-Tek, USA) and commercially available dog-specific ELISA kits in accordance with the manufacturer's instructions were used for the analyses (ER Catalog no: E0414Ca, PR Catalog no: E0058Ca, EGFR Catalog no: E0301Ca, CA 15-3 Catalog no: E0156Ca Bioassay Technology Laboratory, Shanghai, China). Three samples were measured repeatedly in order to determine the coefficients of variance within and between tests. In-test variance coefficients were determined as 3.2%, 2.1%, 3.7%, and 5.2% for ER, PR, EGFR, and CA 15-3 parameters, respectively. The variance coefficients between the tests were found to be less than 10%.

Statistical analysis

A priori power sample size analysis was performed by G*Power version 3.1.9.7 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Calculations revealed that a total sample size of n = 123 would be sufficient to provide 98% power with 0.05 α-error probability and an effect size of 0.40.

The SPSS 13.0 program (IBM, New York, USA) was used for statistical analysis. First, normal distribution of the data was analyzed by the Shapiro Wilk test. The relationship between parameters (ER, PR, EGFR, CA 15-3) was evaluated by the Pearson correlation test. One-way ANOVA and Duncan tests were used to determine the differences between the study groups. A t-test was performed to determine the difference between the invasion status of the masses in the group MET. One-way Anova and Duncan tests were used to determine the differences between histopathological subtypes, histopathological grade, and mass sizes of group MET. In addition, the significance of the distribution of histopathological stages to the mammary lobes in the MET group was evaluated by the Kruskal-Wallis test. The significance level was considered as $P < 0.05$.

Results

The changes in the amount of ER, PR, EGFR, and CA 15-3 were evaluated in terms of various histopathological and clinical findings, and the obtained results were presented in Figure 1, Figure 2, Figure 3, and Figure 4, respectively.

Comparison of the groups and histological findings related to the evaluated biomarkers

The amounts of ER and PR were significantly higher in the BMT group (10.33± 0.76 and 17.53± 1.68 ng/ml) than in the MET group (8.43± 0.30 and 13.81± 0.55 ng/ml) (P<0.05) and in the H&D group (8.53± 0.32 and 13.69± 0.61 ng/ml) (P<0.05) (Figure 1 and Figure 2). While there was no significant difference between the study groups in terms of EGFR amounts (P>0.05), CA 15-3 levels were found to be significantly higher in the MET group (1.27± 0.07 ng/ml) compared to the H&D group (0.91± 0.08 ng/ml) (P=0.01) (Figure 3 and Figure 4). In this study, ER was moderately and positively correlated with PR (r=0.383, P<0.001), while it was low and positively correlated with EGFR (r=0.223, P<0.05). Also, CA 15-3 was moderately and positively correlated with EGFR (r=0.376, P<0.001), while it was low and positively correlated with PR (r=0.216, P<0.05) (Table 2). Histological subtypes of group MET were simple carcinoma (n=31), solid carcinoma (n=5), carcinoma complex type (n=13), carcinoma mixed type (n=7), carcinoma specific type (n=7), carcinoma *in situ* (n=1), ductal carcinoma (n=1), and intraductal papillary carcinoma (n=1). Histopathological subtypes with one case each were not included in the statistical evaluation. There was no significant difference in the evaluated ER, PR, EGFR, and CA15-3 parameters according to the histopathological subtypes (P>0.05). Histopathological grading and their corresponding numbers in group MET were Grade 1 (n=23), Grade 2 (n=30), and Grade 3 (n=13) according to histopathological grade. There was no significant difference between histopathological grades in relation to ER, PR, EGFR, and CA15-3 parameters in group MET (P > 0.05).

Table 2: Correlation coefficients (R) of ER, PR, EGFR and CA 15-3 parameters

	ER	PR	EGFR	CA 15-3
ER	1	.383**	.223*	.139
PR		1	.167	.216*
EGFR			1	.376**
CA 15-3				1

R: Pearson correlation coefficient, ** Correlation is important at the level of 0.01. * Correlation is significant at the level of 0.05

Comparison of clinical aspects related to the evaluated biomarkers

Mass size was evaluated in accordance with TNM classification in the MET group. Accordingly, tumors were grouped as 0-3 cm (T1, n=37), 3-5 cm (T2, n=13), and larger than 5 cm (T3, n=16). There was no significant difference in ER, PR, EGFR, and CA15-3 parameters according to mass size (P > 0.05). On clinical and histopathological examination, 18 of the tumors in the MET group were found to have invaded the muscles or inguinal intramammary lymph nodes, and 48 of them were non-invasive. Accordingly, there was no significant difference in ER, PR, EGFR, and CA 15-3 parameters (P > 0.05) in terms of the presence of the invasion. The relevant mammary lobes of the masses in Group MET were determined as axillary lobe (n=5), thoracic lobe (n=6), cranioabdominal lobe (n=16), caudoabdominal lobe (n=21), and inguinal lobe (n=19). The effect of tumor localization on histopathological grade in this group was evaluated. In our study, although tumors were more localized in the last 3 mammary lobes, there was no significant difference between histopathological grades and tumor localization (P>0.05).

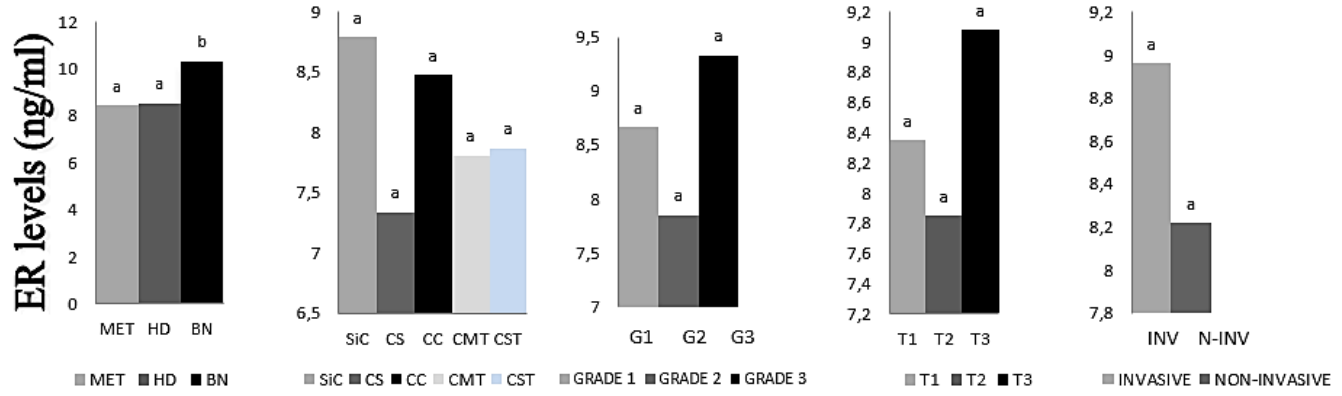

Figure 1: ER levels among histopathological and clinical groups and their significance. MET: Malignant epithelial tumor, HD: Hiperplasia and dysplasia, BN: Bening neoplasms, SiC: Simple carcinoma, CS: Solid carcinoma, CC: Complex carcinoma, CMT: Carcinoma mix type, CST: Carcinoma special type, INV: Invasive, N-INV: Non-invasive. [a,b] Different letters in each graph indicate the significance (P<0.05)

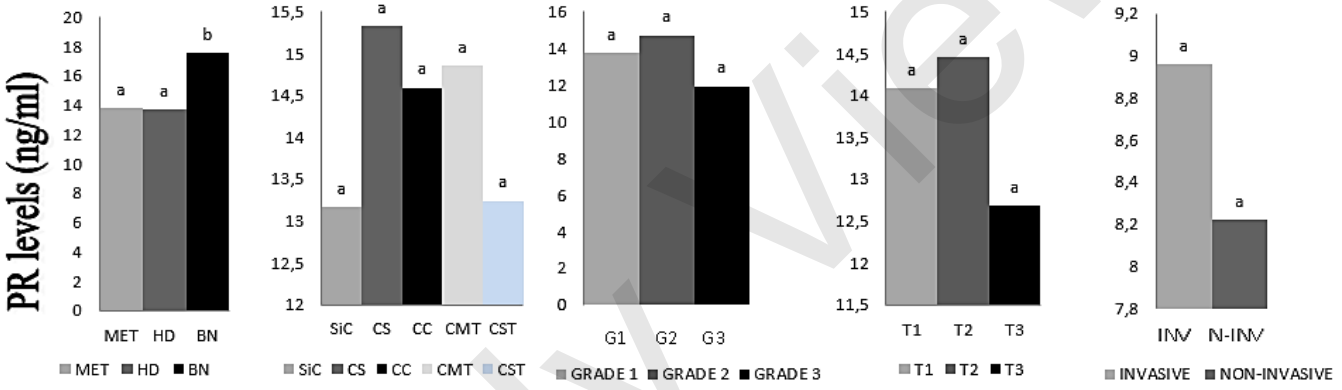

Figure 2: PR levels among histopathological and clinical groups and their significance. MET: Malignant epithelial tumor, HD: Hiperplasia and dysplasia, BN: Bening neoplasms, SiC: Simple carcinoma, CS: Solid carcinoma, CC: Complex carcinoma, CMT: Carcinoma mix type, CST: Carcinoma special type, INV: Invasive, N-INV: Non-invasive. [a,b] Different letters in each graph indicate the significance (P<0.05)

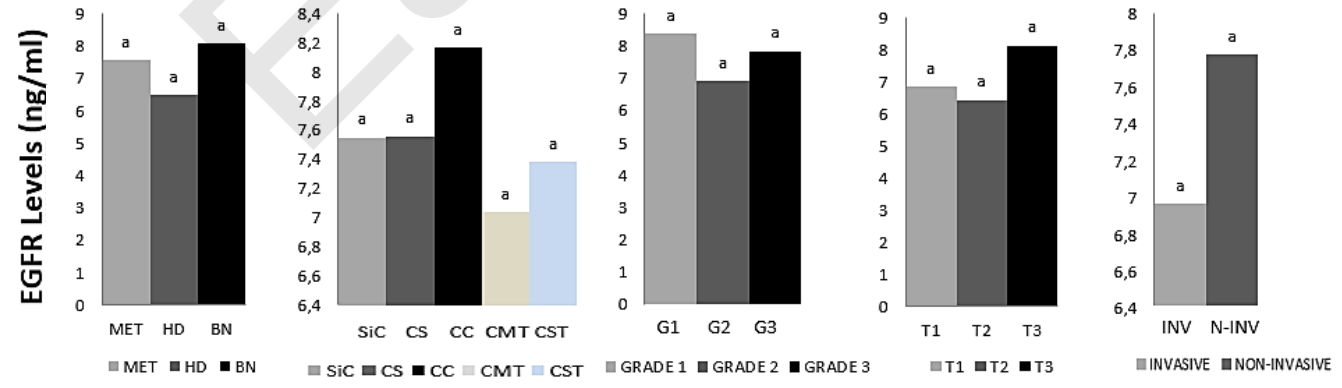

Figure 3: EGFR levels among histopathological and clinical groups and their significance. MET: Malignant epithelial tumor, HD: Hiperplasia and dysplasia, BN: Bening neoplasms, SiC: Simple carcinoma, CS: Solid carcinoma, CC: Complex carcinoma, CMT: Carcinoma mix type, CST: Carcinoma special type, INV: Invasive, N-INV: Non-invasive. [a,b] Different letters in each graph indicate the significance (P<0.05)

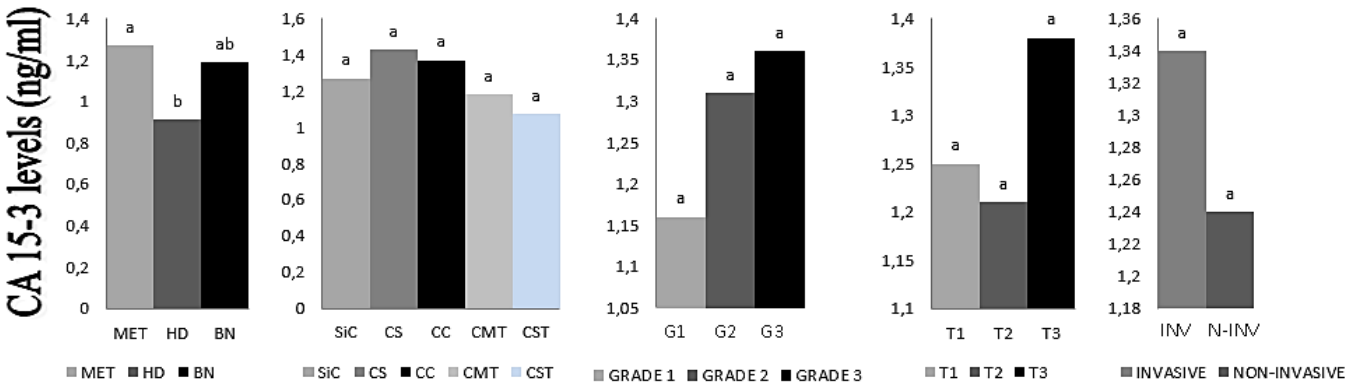

Figure 4: CA15-3 levels among histopathological and clinical groups and their significance. MET: Malignant epithelial tumor, HD: Hiperplasia and dysplasia, BN: Bening neoplasms, SiC: Simple carcinoma, CS: Solid carcinoma, CC: Complex carcinoma, CMT: Carcinoma mix type, CST: Carcinoma special type, INV: Invasive, N-INV: Non-invasive. [a,b] Different letters in each graph indicate the significance (P<0.05)

Discussion

Biomarkers are biological molecules that can be measured in blood or tissues and provide information for the presence of a disease (6). In accordance with the previous report, the difference of ER, PR, EGFR, and CA15-3 biomarkers in canine mammary tissue with different pathologies and the change in amounts of the specified biomarkers in malignant epithelial tumors related to clinical parameters were investigated in this study. High surface membrane progesterone expression was determined in benign tumors (22). The researchers (22) indicated that the proportion of progesterone-containing cells in malignant tumors (13.63±3.5) was lower than in benign tumors (39.28±5.18). Inconsistent with the previous report, PR levels in BMT were significantly higher than in group MET ($P < 0.05$). Millanta et al. (23) investigated the PR in hyperplasia/dysplasia and benign and malignant mammary tumors, and they found the lowest PR levels in malignant mammary tumors. According to the data obtained, both studies are compatible with each other. In the present study, it was found that the amount of ER was statistically significantly higher in benign tumors than in malignant epithelial tumors and hyperplasia&dysplasia groups ($P < 0.05$). De Las Mulas et al. (24) also found the amount of ER to be significantly higher in benign tumors in their study with mammary tissue. When the malignancy increases, the amount of estrogen hormone also increases. As the amount of hormone increases, the rate of destruction of the receptors cannot reach the rate of construction, and when the amount of estrogen is high, the down-regulation of ER occurs (25). The low ER levels in the malignant tumor in this study are thought to be due to down-regulation. Consistent with the researcher report (9), the positive correlation between ER and PR was observed in this study. Accordingly, it is thought that the use of steroid receptors in canine mammary tumors would be appropriate from a diagnostic and/or pathognomonic point of view. It was hypothesized that the progression towards malignancy in canine mammary tumors therefore appears to be associated with a decrease in steroid hormone dependency.

Researchers have reported that the presence of a direct correlation between EGFR and ER concentrations in malignant mammary tumors in dogs may be associated with an estrogen-dependent expression of EGFR or a similar pattern of regulation of receptors (26). In this study, there is a positive correlation between ER and EGFR, in agreement with the researchers (26, 27). This situation is thought to be due to the synergistic effects of ER and EGFR in mammary tumors, as stated in many studies (26, 27). In the present study, no statistically significant difference was found in terms of EGFR in benign tumors, malignant epithelial tumors, and H&D groups ($P > 0.05$). Gamma et al. (28) found higher EGFR levels in malignant mammary tumors than in benign mammary tumors. As the reason for this contrast, it is thought that the growth of the tissues continues in the mammary tissues with hyperplasia & dysplasia and benign tumors, as in the malignant epithelial mammary tumors, and because EGFR is a part of this growth, no difference was detected between the groups. In this study, the CA 15-3 level in malignant epithelial tumors was found to be significantly higher compared to the H&D group ($P=0.01$). Jain et al. (29) reported that CA 15-3 is at high levels in malignant mammary tumors. Ramadan et al. (30) found that serum CA 15-3 levels are higher in malignant mammary tumors compared to healthy mammary tissue. Benign and malignant tumors can coexist in the same animal. Since the present study was based on mammary lobes, benign and malignant tumors may be present in close lobes. Since the CA 15-3 biomarker is also a biomarker measured in blood serum, it can pass from one lobe to the other via the hematogenous way (31). Therefore, it is thought that there is no significance between benign and malignant tumors. Sustained elevation of CA 15-3 in breast cancer cases in humans has been associated with the human epidermal growth factor receptor (HER2) positivity (32). In this study, a positive correlation was found between CA 15-3 and EGFR ($P<0.001$). This is thought to

be due to the important role of both parameters in tumor pathogenesis.

In this study, ER and EGFR amounts were not statistically different related to the histological subtypes in group MET (P>0.05). Millanta et al. (33) found no significant difference in ER levels between the histological subtypes of canine mammary tumors. In addition, although different materials or different analysis methods were used in dogs with mammary tumors, researchers (14, 28, 34) did not detect a statistical difference in the amount of EGFR related to tumor subtypes. In the present study, no significant difference was detected in the amount of CA 15-3 in terms of the histological subtypes (P > 0.05). Manual et al. (15) investigated CA 15-3 levels in canine mammary tissue, and they did not observe the significance in CA 5-3 levels in terms of histological subtypes of the tumor.

Histopathological grading is an evaluation to obtain information about the tumor's malignancy, extent of spread, and metastatic potential (35). The differences of the biomarkers evaluated in this study according to the histological grades of the tumor were examined. In the present study, no significant differences were observed in the ER and PR parameters related to the histological grades of the tumor (P>0.05). Gama et al. (28) stated that EGFR levels did not differ in regard to the histological grading. Günay Uçmak et al. (34) reported that the amount of EGFR in the circulation was higher in histopathological grade 2 tumors than in grade 1 tumors. The opposite result with the previous reports (28, 34) is thought to be due to the fact that this study material is tissue. In this study, CA 15-3 levels were not different related to tumor grades (P>0.05). Shao et al. (31) reported that the amount of CA 15-3 tended to increase with tumor grade. The researchers (15, 36) reported higher CA 15-3 levels in grade 2 and grade 3 carcinomas. The inconsistency with the studies is thought to be due to the determination of CA 15-3 from serum in the compared studies and the difference in the number of cases belonging to malignancy grades.

Tumor size is one of the important pathognomonic factors in malignant mammary tumors (37). When the biomarkers in this study were evaluated according to tumor size, no significant difference was found in ER levels in terms of tumor size (P>0.05). This is thought to be due to the fact that ER down-regulation does not show any difference in mass size in malignant mammary tumors. In the present study, no significance was determined in PR amounts related to tumor size (P>0.05). De Las Mulas et al. (37) found that tumors with PR were smaller in size. Compared to the small-sized tumors used in previous studies, the tumors in this study were less numerous, and this is thought to be the reason for the contradictory results. In the present study, no difference was detected in EGFR levels in regard to different tumor sizes (P>0.05). The researchers (38, 39) reported that as the tumor size increases, the amount of EGFR also increases. The difference between studies can be explained by the fact that EGFR down-regulates its own receptor as the mass grows (28). Because elevated CA 15-3 levels are associated with large tumor size and malignancy, tumor malignancy is assessed by the measured amounts of CA 15-3 (36). In this study, no statistically significant difference was found between the mass sizes in terms of the amount of CA 15-3 (P>0.05). The reason for this is thought to be high CA 15-3 levels in malignant epithelial tumors, regardless of mass size.

In the present study, ER levels were not different in regard to the invasion status of the tumors (P>0.05). The researchers (23, 40) found the significant differences in ER levels related to the invasion status of canine mammary tissue (P < 0.01, P = 0.05, respectively). In this study, PR levels did not differ depending on the invasion status of the tumors (P>0.05). Also, Millanta et al. (33) found no significance in PR levels in regard to the tumor invasion in their study using both cat and dog mammary tissue. Adhesion to the skin and underlying tissues increases EGFR levels (20). In the present study, EGFR levels did not differ significantly according to tumor invasion status (P>0.05). Gama et al. (28) found no significance in EGFR levels in terms of the tumor invasion. The discrepancies in EGFR amounts are thought to be due to the difference in the number of invasive tumor tissues studied. Levels of CA 15-3 increase with invasion (41). Although the amount of CA 15-3 was found to be high in invasive malignant epithelial tumors in this study, it could not reach significance (P > 0.05). This difference can be explained by the increase in CA 15-3 levels with increasing metastasis and histological grade and the fact that CA 15-3 levels in malignant epithelial tumors are not affected by the mass size or by the muscle invasion.

Canine mammary tumors appear to be predominantly localized in the inguinal lobes, followed by the caudoabdominal lobes. The malignant epithelial tumors in this study were mostly in the inguinal lobes, which is consistent with a previous study (42). Although the localization of malignant tumors was more than posterior mammary lobes, no relation was found between tumor localization and histological grading in this study. Our findings show that ER and PR biomarkers can distinguish benign from malignant and benign from H&D. A significant rise in ER and PR in BMT tissues exhibited the need for steroid hormones in tumor-growing tissues. In addition, the CA 15-3 biomarker can distinguish between malignant and H&D. Although these biomarkers could not make any distinction in terms of clinical and pathological findings (histological subtypes, histological grades, tumor size, invasion status, and localization) in malignant tumors, it was concluded that they were capable of revealing the tumor formation.

Conclusion

In conclusion, this study demonstrated that tissue tumor markers CA15-3, ER, and PR can be used as diagnostic tools for the screening of canine mammary tumors. This study stated that ELISA is capable enough to detect the ER, PR, EGFR, and CA 15-3 in canine mammary tumor tissue. The results obtained suggest that the combined detection of tumor biomarkers CA15-3, ER, and PR can be used as a method for the diagnosis

of canine mammary tumors and may increase the rate of diagnosis. ER and PR can distinguish benign tumors from malignant tumors and H&D, while CA 15-3 can distinguish malignant tumors from H&D.

Acknowledgements

Any additional data supporting this study are available from the authors (Ç.N.K., Ç.T., Z.G.U., A.A.) upon reasonable request. English editing of the manuscript was performed by 'QuillBot'.

Conflıct of ınterest. The authors declare no conflict of interest.

Fundıng statement. This study was supported by Istanbul University-Cerrahpaşa Scientific Research Projects Coordination Unit. Project number: TDK-2020-34419.

References

1. Bronden LB, Flagstad A, Kristensen AT. Veterinary cancer registry in companion animal cancer; a review. Vet Comp Oncol 2007; 5(3): 133–44.

2. Šoštarić-Zuckermann IC, Severin K, Hohšteter M, et al. Incidence and types of canine tumours in Croatia. Vet Arh 2013; 83(1): 31–45.

3. Sarver AL, Makielski KM, DePauw TA, Schulte AJ, Modiano JF. Increased risk of cancer in dogs and humans: A consequence of recent extension of lifespan beyond evolutionarily determined limitations? Aging Cancer 2022; 3(1): 3–19.

4. Zuchi TLVL, Lopatini CL, Faria JLM. Veterinary approaches to canine mammary tumors and knowledge of the consensus statement in Brazil. Braz J Vet Pathol 2021; 14: 24 – 28.

5. Nikodinovska T, Gombač M, Dolenšek T, Tekavec K, Šturm S, Cvetko M, Pavlin K, Fras DM, Švara T. Incidence and types of canine tumours in Slovenia (2000-2020): A Retrospective study. Slovenian Veterinary Research 2025; 62(27): 27-39.

6. Henry JC. Biomarkers in veterinary cancer screening: applications, limitations and expectations. Vet J 2010; 185: 10–14.

7. Mobasheri A, Cassidy J. Biomarkers in veterinary medicine: towards targeted, individualised therapies for companion animals. Vet J 2010; 185: 1–3.

8. Sturgeon CM, Lai LC, Duffy MJ. Serum tumour markers: how to order and interpret them. Br Med J 2009; 339: 852-8.

9. Kim NH, Lim KY, Im KS. Evaluation of clinicopathological characteristics and oestrogen receptor gene expression in oestrogen receptor-negative, progesterone receptor- positive canine mammary carcinomas. J Comp Pathol 2014; 151: 42–50.

10. Queiroga FL, Perez-Alenza D, Gonzalez-Gil A, Silvan G, Peña L, Illera JC. Serum and Tissue Steroid Hormone Levels in Canine Mammary Tumours: Clinical and Prognostic Implications. Reprod Dom Anim 2015; 50: 858–865.

11. Shah R, Rosso K, Nathanson SD. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World J Clin Oncol 2014; 5: 283-298.

12. Mc Cormack O, Harrison M, Kerin MJ, McCann A. Role of the progesterone receptor (PR) and the PR isoforms in breast cancer. Crit Rev Oncog 2007; 13: 283-301.

13. Kim JH, Im KS, Kim NH. Expression of HER-2 and nuclear localization of HER-3 protein in canine mammary tumors: histopathological and immunohistochemical study. Vet J 2011; 189: 318–22.

14. Carvalho MI, Guimarães MJ, Pires I, et al. EGFR and microvessel density in canine malignant mammary tumours. Res Vet Sci 2013; 95: 1094–1099.

15. Manuali E, De Giuseppe A, Feliziani F, et al. CA 15-3 cell lines and tissue expression in canine mammary cancer and the correlation between serum levels and tumour histological grade. BMC Vet Res 2012; 8: 86-98.

16. Hollinghsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev Canc 2004; 4: 45–60.

17. Martin A, Corte MD, Alvarez AM, Rodriguez JC, Andicoechea A, Bongera M, Junquera S, Pidal D, Allende T, Muñiz JL, Vizoso F. Prognostic value of pre-operative CA 15.3 levels in breast cancer. Antican. Res 2006; 26(5B): 3965–3971.

18. Agrawal AK, Jelen M, Rudnicki J, Grzebieniak Z, Zyśko D, Kielan W, Słolina J, Marek G. The importance of preoperative elevated serum levels of CEA and CA 15–3 in patients with breast cancer in predicting its histological type. Folia Histochem Cytobiol 2010; 48(1): 26–29.

19. Illera JC, Perez-Alenza MD, Nieto A, et al. Steroids and receptors in canine mammary cancer. Steroids 2006; 71: 541-48.

20. Queiroga FL, Perez-Alenza MD, González-Gil A, Silván G, Peña L, Illera JC. Quantification of epidermal growth factor receptor (EGFR) in canine mammary tumours by ELISA assay: clinical and prognostic implications. Vet Comp Oncol 2017; 15: 383–390.

21. Owen LN. TNM Classification of tumors in Domestic Animals, World Health Organization, Geneva, Switzerland, 1980.

22. Thuroczy J, Reisvaag GJK, Perge E, Tibold A, Szilagyi J, Balogh L. Immunohistochemical detection of Progesterone and Cellular Proliferation in Canine Mammary Tumors. J Comp Pathol 2007; 137: 122-129.

23. Millanta F, Calandrella M, Bari G, Niccolini M, Vannozzi I, Poli A. Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. Res Vet Sci 2005; 79: 225-232.

24. De Las Mulas JM, Van Niel M, Millán Y, Blankenstein MA, Van Mil F, Misdorp W. Immunohistochemical analysis of estrogen receptors in feline mammary gland benign and malignant lesions: comparison with biochemical assay. Domest Anim Endocrinol 2000; 18: 111-125.

25. Hatsumi T, Yamamuro Y. Downregulation of estrogen receptor gene expression by exogenous 17beta-estradiol in the mammary glands of lactating mice. Exp Biol Med 2006; 231: 311-316.

26. Donnay I, Devleeschouwer N, Wouters-Ballman P, Leclercq G, Verstegen J. Relationship between receptors for epidermal growth factor and steroid hormones in normal, dysplastic and neoplastic canine mammary tissues. Res Vet Sci 1996; 60: 251–254.

27. Levin ER. Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. Mol Endocrinol 2003; 7: 309–317.

28. Gama A, Gärtner F, Alves A, Schmitt F. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in canine mammary tissues. Res Vet Sci 2009; 87: 432–437.

29. Jain M, Ingole SD, Deshmukh RS, et al. CEA, CA 15-3, and miRNA expression as potential biomarkers in canine mammary tumors. Chromosome Res 2021; 29: 175–188.

30. Ramadan ES, Salem NY, Emam IA, AbdElKader NA, Farghali HA, Khattab MS. MicroRNA 21 expression, serum tumor markers, and immunohistochemistry in canine mammary tumors. Veter Res Commun 2021; 46: 377-388.

31. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. Plos One 2015; 10: 1–11.

32. Hashim ZM. The significance of CA15-3 in breast cancer patients and its relationship to HER-2 receptor status. Int J Immunopathol Pharmacol 2014; 27: 45–51.

33. Millanta F, Calandrella M, Vannozzi I, Poli A. Steroid hormone receptors in normal, dysplastic and neoplastic feline mammary tissues and their prognostic significance. Vet Rec 2006; 158: 821-824.

34. Günay Uçmak Z, Timirci Kahraman Ö, İnal Gültekin G, Değirmencioğlu S, Yaylım İ, Güvenç K. Determining the expression levels of circulating tumour cell markers in canine mammary tumours. Acta Vet Brno 2021; 90: 191–200.

35. Canadas A, França M, Pereira C, et al. Canine mammary tumors: comparison of classification and grading methods in a survival study. Vet Pathol 2019; 56: 208–219.

36. Kaszak I, Ruszczak A, Kanafa S, Kacprzak K, Król M, Jurka P. Current biomarkers of canine mammary tumors. Acta Vet Scand 2018; 60: 66.

37. De Las Mulas JM, Millán Y, Dios R. A Prospective Analysis of Immunohistochemically Determined Estrogen Receptor and Progesterone Receptor Expression and Host and Tumor Factors as Predictors of Disease-free Period in Mammary Tumors of the Dog. Vet Pathol 2005; 42: 200–212.

38. Vogl G, Bartel H, Dietze O, Hauser-Kronberger C. HER2 is unlikely to be involved in directly regulating angiogenesis in human breast cancer. Appl Immunohistochem Mol Morphol 2006; 14: 138–145.

39. Kallel I, Khabir A, Boujelbene N, et al. EGFR overexpression relates to triple negative profile and poor prognosis in breast cancer patients in Tunisia. J Recept Signal Transduct Res 2012; 32: 142–149.

40. Mainenti M, Rasotto R, Carnier P, Zappulli V. Oestrogen and progesterone receptor expression in subtypes of canine mammary tumours in intact and ovariectomised dogs. Vet J 2014; 202: 62–68.

41. Campos LC, Lavalle GE, Estrela-Lima A, et al. CA15.3, CEA, and LDH in Dogs with Malignant Mammary Tumors. J Vet Intern Med 2012; 26: 1383–1388.

42. Günay Uçmak Z, Güvenç K. Malign Meme Tümörlü Dişi Köpeklerde Klinik ve Bazı Hematolojik Parametreler Arasındaki İlişkinin Değerlendirilmesi. Türkiye Klinikleri J Vet Sci 2019; 10: 45-52.