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Assessment of Thrombocyte, Osteopontin and Vascular Endothelial Growth Factor Receptor Levels in Bitches with Inflammatory or Ulcerative Mammary Tumors: Preliminary Study-Methodological Study

İnflamatuar veya Ülseratif Meme Tümörlü Köpeklerde Trombosit, Osteopontin ve Vasküler Endotel Büyüme Faktörü Reseptörü Seviyelerinin Değerlendirilmesi: Ön Çalışma-Metodolojik Çalışma

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ABSTRACT Objective: The aim of this preliminary study is to compare the histological grading, tumor-lymph node-metastasis (TNM) scores, platelet (PLT) levels, serum/plasma osteopontin (OPN) and vascular endothelial growth factor (VEGF) between ulcerative or inflammatory and non-ulcerative and non-inflammatory malignant mammary tumors of dogs. **Material and Methods:** Study groups were formed as; ulcerated mammary tumors (UMT; n=5), inflammatory mammary tumors (IMT; n=6) and non-ulcerative and non-inflammatory mammary tumors (MT; n=7). **Results:** TNM scores in UMT and IMT groups were significantly higher than MT group ($p<0.05$ and $p<0.01$; respectively). Levels of PLT were tended to be higher in group UMT than in group MT, but failed to reach significance ($p=0.08$). Plasma OPN levels were significantly higher in group IMT than in group MT ($p<0.05$). Serum and plasma VEGF levels did not differ between the groups ($p>0.05$). Plasma OPN was moderately correlated with serum OPN ($r=0.562$, $p<0.05$). Serum OPN levels were positively correlated with histological grading of the tumor ($r=0.518$, $p<0.05$). VEGF was not associated with ulceration or inflammation in mammary tumors due to the malignant character in all groups. However, the plasma OPN level was predictive in the presence of inflammation on tumor while serum OPN levels were significantly associated with histological grade of the tumor. **Conclusion:** In conclusion, PLT levels and OPN levels are capable to reflect the increased malignancy as in histological grades and TNM scores in female dogs with canine mammary tumor. As a result of that OPN could have a pathophysiological role in canine mammary tumors as in human breast cancer.

Keywords: Inflammation; osteopontin; platelet; ulceration; vascular endothelial growth factor

ÖZET Amaç: Bu ön çalışmanın amacı, köpeklerde ülseratif, inflamatuar ve ülseratif/inflamatuar olmayan malign meme tümörleri arasında histolojik derecelendirme, tümör-lenf nodu-metastaz (TNM) skorları, trombosit [platelet (PLT)] düzeyleri, serum/plazma osteopontin (OPN) ve vasküler endotelyal büyüme faktörü [vascular endothelial growth factor (VEGF)] düzeylerini karşılaştırmaktır. **Gereç ve Yöntemler:** Çalışma grupları; ülserli meme tümörleri (ÜMT; n=5), inflamatuar meme tümörleri (İMT; n=6) ve ülseratif olmayan ve inflamatuar olmayan meme tümörleri (MT; n=7) olarak oluşturuldu. **Bulgular:** ÜMT ve İMT gruplarında TNM skorları, MT grubundan anlamlı olarak yüksekti (sırasıyla $p<0,05$; $p<0,01$). PLT düzeyleri, grup MT'ye kıyasla grup ÜMT'de daha yüksek olma eğilimindeydi, ancak anlamlı düzeye ulaşamadı ($p=0,08$). Plazma OPN düzeyleri, grup İMT'de grup MT'ye göre anlamlı derecede yüksekti ($p<0,05$). Serum ve plazma VEGF düzeyleri gruplar arasında farklılık göstermedi ($p>0,05$). Plazma OPN, serum OPN ile orta düzeyde korelasyon gösterdi ($r=0,562$; $p<0,05$). Serum OPN düzeyleri, tümörün histolojik derecesi ile pozitif korelasyon gösterdi ($r=0,518$; $p<0,05$). Tüm gruplardaki malign karakter nedeniyle VEGF, meme tümörlerinde ülserasyon veya inflamasyon ile ilişkili değildi. Ancak plazma OPN düzeyi, tümörde inflamasyon varlığında öngörücüydü, serum OPN düzeyleri ise tümörün histolojik derecesi ile anlamlı derecede ilişkiliydi. **Sonuç:** Sonuç olarak PLT düzeyleri ve OPN düzeyleri, köpek meme tümörü olan dişi köpeklerde histolojik derecelerde ve TNM skorlarında olduğu gibi artan maligniteyi yansıtabilir. Bunun bir sonucu olarak OPN'nin, insan meme kanserinde olduğu gibi köpek meme tümörlerinde de patofizyolojik bir rolü olabilir.

Anahtar Kelimeler: İnflamasyon; osteopontin; trombosit; ülserasyon; vasküler endotelyal büyüme faktörü

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Mammary tumors are most frequent type of tumor in intact female dogs.[1] Malignant mammary tumors are constituted 53.3% of canine mammary tumors.[2] On physical examination, tumors can be detected as small or large, single or multiple, fixed or capsulated, ulcerated or inflammatory.[3] Inflammation in dogs with mammary carcinoma causes edema and pain in mammary glands. Also, prognosis is extremely poor in such cases.[4] Dogs with distant metastasis, invasive, ulcerative and inflammatory mammary carcinoma are quite likely to have coagulopathies.[5] Platelets (PLT) accelerate cancer progression and metastasis.[6] Levels of PLT in dogs with malignant mammary tumors were found higher compared to healthy ones.[7]

Osteopontin (OPN) is a 35kDa glycophosphoprotein which plays an important role in diagnosis and treatment of cancers.[8,9] It participates in biomineralization, chronic inflammation and tumor biology (invasion, angiogenesis, metastasis, tumor growth and development).[10] Levels of OPN in benign, malignant and normal non-secretory breast epithelia are investigated and OPN is undetectable in humans with normal breast epithelia.[11] Also, OPN is overexpressed in metastatic cases which are strongly associated with poor prognosis in human breast cancer.[12]

Vascular endothelial growth factor (VEGF) is a protein which controls angiogenesis.[13] It has a major role on formation of new vessels and stimulation of cell migration.[14,15] Queiroga et al. reported that VEGF is considered as a biomarker of tumor development and metastasis because its serum levels are high in infiltrative and necrotic tumors.[16] Besides, VEGF and OPN can function in synergy that they may support cell migration and angiogenesis.[17]

The purpose of this preliminary study is to compare the histological grading, tumor-lymph node-metastasis (TNM) scores, levels of PLT, serum/plasma OPN and VEGF in ulcerative or inflammatory and non-ulcerative and non-inflammatory malignant mammary tumors of dogs. In this study, it is also aimed to evaluate the relationship between the circulating VEGF and OPN levels and tumor progression in mammary tumors with necrosis or inflammation.

MATERIAL AND METHODS

ETHICAL STATEMENT

All animal procedures were carried out in accordance with the approval of the Animal Experiments Unit Ethics Committee at İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine (date: August 1, 2019; no: 2019/37). The owners' consent was obtained for each bitch.

All animals in the study were treated humanely in accordance with the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html) and the relevant Experimental Animals Ethics Committee Approval Report was obtained.

ANIMALS AND STUDY DESIGN

The preliminary materials of the study were 30 bitches with mammary tumors presented to Animal Hospital of İstanbul University-Cerrahpaşa. They were clinically examined (inspection and palpation) with regard to the presence or absence of inflammation and/or ulceration. On clinical examination, palpation of axillar and popliteal lymph nodes was performed. All bitches were treated surgically (total bilateral mastectomy) after the routine hematological and radiographic examinations. Three-view thoracic radiographs were taken to determine the metastasis on lungs by digital radiography (DR, Ekovia, Korea). Excised mammary glands of the bitches were prepared for histopathological analysis as described previously.[18] The groups consisted of malignant mammary tumors with an epithelial origin. The bitches with malignant mesenchymal tumors or benign tumors and the bitches with both inflammation and ulceration together in a mammary tumor were not included to the study. In addition, intra-mammary lymph nodes were also examined during the histopathological analysis of the total mastectomy material. This study consisted of 18 bitches with malignant mammary tumors; 5 of which were only ulcerated, 6 of which were only inflammatory, and 7 of which were neither ulcerated nor inflammatory. Study groups were formed according to clinical appearance as; group ulcerated mammary tumors (UMT), group inflammatory mammary tumors (IMT) and group

non-ulcerative and non-inflammatory mammary tumors (MT).

LABORATORY ANALYSIS

Blood was collected by the puncture of jugular vein and collected into one clot separator tube and one EDTA containing tube prior to surgery. They were centrifuged at 4 °C and 3,500 g for 15 minutes in order to obtain the serum and plasma samples. All samples were stored at -20 °C until the analysis. Levels of OPN and VEGF in serum and plasma were determined with commercially available canine specific enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (OPN Catalogue no: 201-15-0618, VEGF Catalogue no: 201-15-2415, Sunred Biological Technology, Shangai, China). Assay sensitivity range for VEGF and OPN were 3-900 ng/L and 0.25-70 ng/mL, respectively. The blood collected into EDTA containing tubes was used to determine total number of PLT. The levels of PLT were obtained from the results of routine pre-operative hematological analysis (Procyte Dx Hematology Analyzer, Idexx, USA). The reference range of PLT count was 110-460 x103/µL for dogs.

HISTOPATHOLOGICAL ANALYSIS

Tumor samples were histopathologically examined for staging and determination of inflammation or ulceration. Mammary tumors were classified histologically according to Goldschmidt et al. as grade 1, 2 and 3. scoring of mammary tumors was determined according to TNM staging system.[19,20]

STATISTICAL ANALYSIS

Before the study, a power analysis was performed using G*Power 3.1.9.7 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Calculations revealed that total sample size of n=18 would be sufficient to provide 80% power with 0.05 α-error probability. Statistical analyses were performed with SPSS 13.0 package program (SPSS Inc, Chicago, Illinois, USA). Normal distribution of data was checked by using the Shapiro-Wilk test. Non-parametric tests were used because the data did not show normal distribution. Groups were compared with regard to histological gradings, TNM scores, PLT, plasma/serum OPN and VEGF levels using the Kruskal-Wallis test and the Mann-Whitney U test. Spearman's rho correlation test was used to examine the relationships among the evaluated parameters. Results are expressed as mean±standard error of mean. Statistical significance was set at $p<0.05$.

RESULTS

The mean age of the dogs with malignant mammary tumors was 11.36±0.94 years. Grossly, UMTs were observed as swollen, necrotic, and dark red colored tumors, besides those entities had a tissue loss (Figure 1A). The IMTs were also congested and dark masses, but the epidermis was intact (Figure 1B). Microscopically, UMT group had epidermal and dermal edema, hemorrhage necrosis in various degrees besides necrotic tumoral tissues and inflammatory cells such as macrophages, polymorph leukocytes and mononuclear inflammatory cells

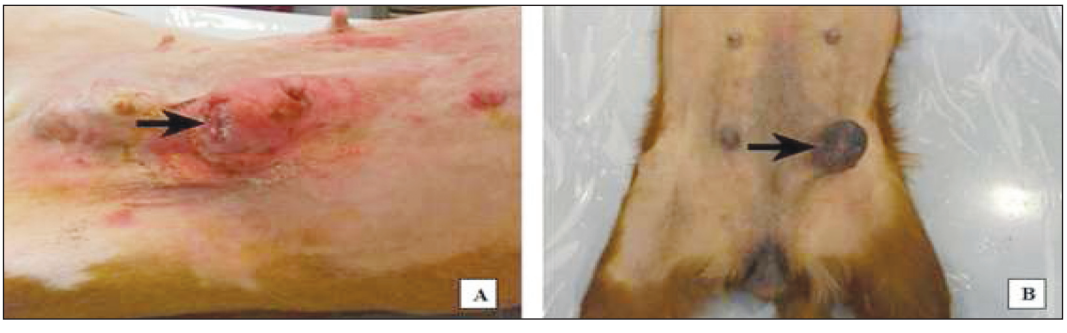

FIGURE 1: Appearances of mammary tumors. **A)** Gross appearance of an ulcerative tumor in the 4th left mammary gland (arrow). **B)** Gross appearance of an inflammatory tumor which is swollen and reddish dark gray color in the 5th left mammary gland (arrow)

(Figure 2A, Figure 2B). In IMT group lymphocytes, plasma cells and macrophages scattered among tumoral foci were observed. Different tumoral patterns such as tubulopapillary or complex carcinomas were detected in the MT group (Figure 2C, Figure 2D).

Numbers of the dogs regarding histological grading and TNM scores are mentioned in Table 1. Histological grading in IMT group had tended to be higher than MT group but failed to achieve significance (p=0.07). However, TNM scores in UMT and IMT groups were significantly higher than MT group (p<0.05 and p<0.01; respectively). Levels of PLT, serum/plasma OPN and VEGF levels and their significances in terms of the groups were presented in Figure 3, Figure 4, Figure 5, respectively.

The highest PLT level was observed in group IMT (550.33±92.76 K/µL). Levels of PLT were tended to be higher in group UMT (501.20±63.31 K/µL) than in group MT (346.43±33.80 K/µL) but failed to reach significance (p=0.08). Plasma OPN levels in group IMT (0.96±0.19 ng/mL) were significantly higher than in group MT (0.35±0.07 ng/mL) (p<0.05). Serum OPN levels did not show statistically significant differences within the groups

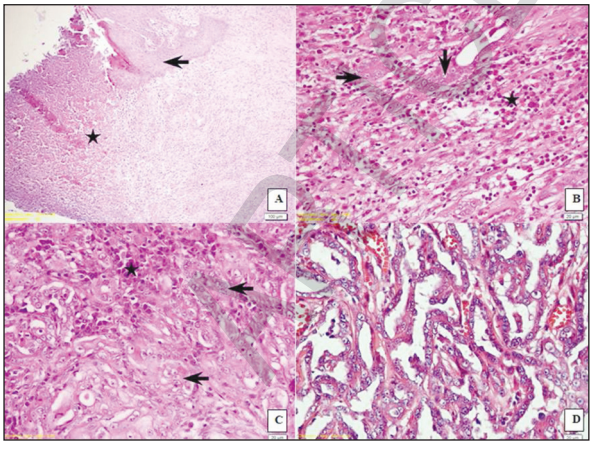

FIGURE 2: Microscopic evaluations of mammary tumors. **A)** Microscopic view of the ulcerative area (star) causing intense inflammation and necrosis in the epidermis (arrow) and dermis in a UMT (H&E, Bar=100 µm). **B)** Tubular arrangements (arrows) and a large number of inflammatory cells (star) infiltrated in tumoral tissue in a UMT (H&E, Bar=20 µm). **C)** Lymphocytic cell (star) infiltration among the tubular structures (arrows) in an IMT (H&E, Bar=20 µm). **D)** Tubular carcinoma area in a mammary gland (H&E, Bar=20 µm); UMT: Ulcerated mammary tumor; H&E: Hematoxylin-eosin; IMT: Inflammatory mammary tumor

TABLE 1: Numbers of the dogs within the groups with regard to histological grading and TNM scores

Groups[1]		UMT (n=5)	IMT (n=6)	MT (n=7)
Grade	1	-	1	4
	2	5	3	3
	3	-	2	-
TNM scores	1	-	-	5
	2	4	4	2
	3	1	2	-

[1]UMT: Ulcerated mammary tumors; IMT: Inflammatory mammary tumors; MT: Non-ulcerative and non-inflammatory mammary tumors; TNM: Tumor-lymph node-metastasis

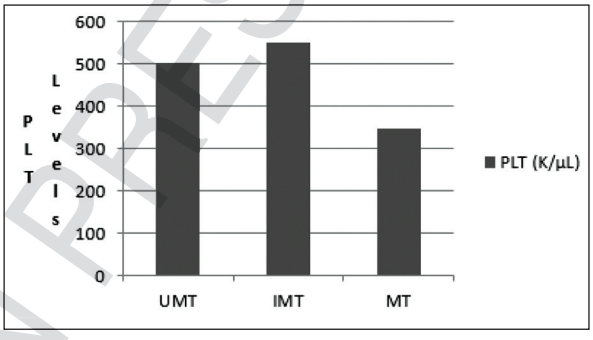

FIGURE 3: PLT levels related to the groups
PLT: Platelet; UMT: Ulcerated mammary tumors; IMT: Inflammatory mammary tumors; MT: Non-ulcerative and non-inflammatory mammary tumors

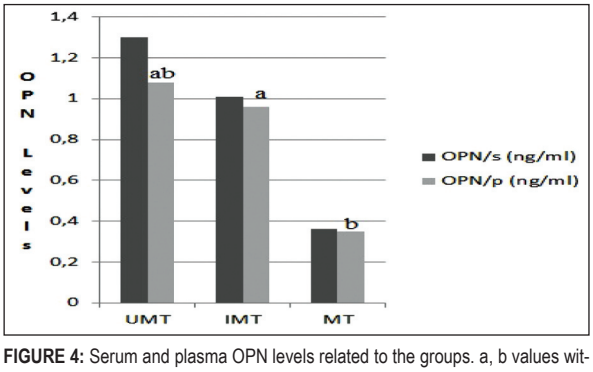

FIGURE 4: Serum and plasma OPN levels related to the groups. a, b values within a row with different superscripts differ significantly at p<0.05
OPN: Osteopontin; OPN/s: Serum OPN; OPN/p: Plasma OPN; UMT: Ulcerated mammary tumors; IMT: Inflammatory mammary tumors; MT: Non-ulcerative and non-inflammatory mammary tumors

(p>0.05). Serum and plasma VEGF levels were not different between the groups (p>0.05). Plasma OPN was moderately correlated with serum OPN (r=0.562; p<0.05). Also, serum OPN levels were moderately correlated with histological grading of the tumors (r=0.518; p<0.05). Serum and plasma VEGF levels

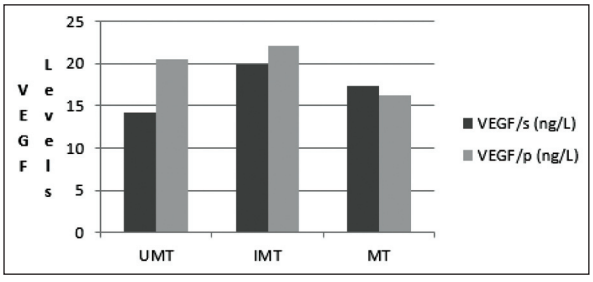

FIGURE 5: Serum and plasma VEGF levels related to the groups
VEGF: Vascular endothelial growth factor; VEGF/s: Serum VEGF; VEGF/p: Plasma VEGF; UMT: Ulcerated mammary tumors; IMT: Inflammatory mammary tumors; MT: Non-ulcerative and non-inflammatory mammary tumors

were not correlated with PLT, TNM, histological grading and serum and plasma OPN levels ($p>0.05$).

DISCUSSION

In this study, the relationship between the circulating VEGF and OPN levels and tumor progression in mammary tumors with necrosis or inflammation were investigated. For this purpose, histological grading, TNM scores, PLT levels, plasma/serum OPN and VEGF levels were evaluated in malignant mammary tumors related to the presence or the absence of inflammation or ulceration. Gupta et al. reported the highest incidence of canine mammary tumors as 10-12 years old. In accordance with the previously reported, the mean age of female dogs with mammary tumors in this study was within the highest incidence ages (11.36±0.94 years).[21]

Histological grading of malignant epithelial canine mammary tumors provides valuable information to clinicians about prognosis and high grade reflects the worsening prognosis.[19] Secretion of large number of cytokines and chemokines exacerbates the tumor inflammation.[22] Elevated levels of cytokines and chemokines were associated with high grade of malignancy in canine mammary tumors.[23] In this study, histological grades of group IMT were tended to be higher than group MT. Even though this study is a preliminary study, the detection of higher histological grades in the presence of inflammation supports the relationship of cytokines and chemokines with malignancy. Scoring of TNM is associated with tumor malignancy and as TNM scores increase, the prognosis gets poorer.[24] Tecles et al. reported that the inflammatory mediators increase in the presence of large masses and ulceration or secondary inflammation of the neoplasms.[25] In this study, increasing TNM score was significantly associated with the presence of ulceration and inflammation. In agreement with the previous reports, presence of ulceration or inflammation on tumor reflected the increased malignancy.[24,25]

Hemostatic changes are frequently seen in dogs with a malignant mammary tumor which have distant metastasis, severe necrosis and inflammation on the tumor. A significant correlation between coagulation parameters and tumor necrosis was observed.[5] In this study, levels of PLT had tendency to be higher in group UMT than group MT. Even if the differences could not reach a significance, elevated levels of PLT in the presence of ulceration on tumor was thought to reflect the increase in malignancy.

OPN plays an important role in tumor progression, inflammation, and metastasis in humans with breast cancer. Tumor cell-secreted OPN has an effective role in the inflammatory process by activating the mammary fibroblasts.[26] In this study, a significant rise in the plasma OPN levels was observed in the group IMT than in the group MT. It was hypothesized that this significant rise supposed to be due to the role of OPN in the inflammatory process in canine mammary tumors as in humans with breast cancer. Although, OPN is related with poor prognosis in human breast cancer, intense expression of OPN is observed in mesenchymal elements of benign mammary tumors than malignant mammary tumors, in canine species.[27,28] Expression of OPN is limited in ulcerated human breast cancer with bone metastases.[29] In this study, the lack of elevated plasma/serum OPN levels in group UMT could be explained by the lack of affinity of OPN to ulcerative masses as reported in human breast cancer.[29]

Inflammation usually occurs in prolonged neoplastic processes. Inflammatory cells secrete some mediators which promote angiogenesis and consequently tumor growth.[23] Queiroga et al. reported that overexpression of cyclooxygenase-2 and VEGF may influence tumor aggressiveness by increasing

angiogenesis.[16] In contrast, the levels of serum and plasma VEGF were not significantly associated with tumor inflammation in this study. It was thought that significant differences were not obtained due to the presence of the same histopathological malignancy type in all groups. In canine mammary tumors, expression of VEGF was investigated in malignant, benign, and non-neoplastic mammary glands.[30-32] Queiroga et al. reported that VEGF expression in malignant tumors was significantly associated with the presence of necrosis.[16] However, other reports showed no significance in VEGF expression with the presence of ulceration and necrosis on the tumor.[30-32] In contrast with Queiroga et al. but in line with the other researchers, serum and plasma VEGF levels were not significantly associated with the presence of ulcerative or necrotic tumor structure in this study.[16,30-32]

In addition to inflammatory process, OPN has a physiological function in tumor biology.[33] Consequently, OPN is potentially valuable biomarker for diagnosing and treating cancers in humans.[10] In human breast cancer, OPN expression correlated with lymph node metastasis, TNM stage, and histological grade.[34] Although all groups in the present study had mammary tumor with malignant character, serum OPN levels were moderately correlated with histological grade. It was hypothesized that similar results could exhibit the role of OPN in the pathophysiology of canine mammary tumor as in human breast cancer.

CONCLUSION

In conclusion, PLT levels and OPN levels are capable to reflect the increased malignancy as in histological grades and TNM scores in female dogs with canine mammary tumor. As a result of that OPN could have a pathophysiological role in canine mammary tumors as in human breast cancer.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Zeynep Günay Uçmak, Lora Koenhemsi;* ***Design:*** *Melih Uçmak, İbrahim Kurban;* ***Control/Supervision:*** *Funda Yıldırım, Hazal Öztürk Gürgen;* ***Data Collection and/or Processing:*** *Zeynep Günay Uçmak, Çağla Nur Küçükbekir;* ***Analysis and/or Interpretation:*** *Lora Koenhemsi, Funda Yıldırım;* ***Literature Review:*** *Zeynep Günay Uçmak, Remzi Gönül;* ***Writing the Article:*** *Lora Koenhemsi, Zeynep Günay Uçmak;* ***Critical Review:*** *Melih Uçmak;* ***References and Fundings:*** *Zeynep Günay Uçmak, Lora Koenhemsi;* ***Materials:*** *Zeynep Günay Uçmak, Funda Yıldırım, Hazal Öztürk Gürgen.*

REFERENCES

1. Sorenmo K. Canine mammary gland tumors. Vet Clin North Am Small Anim Pract. 2003;33(3):573-96. PMID: 12852237
2. Moe L. Population-based incidence of mammary tumours in some dog breeds. J Reprod Fertil Suppl. 2001;57:439-43. PMID: 11787188
3. Hellmén E, Bergström R, Holmberg L, Spångberg IB, Hansson K, Lindgren A. Prognostic factors in canine mammary tumors: a multivariate study of 202 consecutive cases. Vet Pathol. 1993;30(1):20-7. PMID: 8442324
4. Pérez Alenza MD, Tabanera E, Peña L. Inflammatory mammary carcinoma in dogs: 33 cases (1995-1999). J Am Vet Med Assoc. 2001;219(8):1110-4. PMID: 11700710
5. Stockhaus C, Kohn B, Rudolph R, Brunnberg L, Giger U. Correlation of haemostatic abnormalities with tumour stage and characteristics in dogs with mammary carcinoma. J Small Anim Pract. 1999;40(7):326-31. PMID: 10444752
6. Meikle CK, Kelly CA, Garg P, Wuescher LM, Ali RA, Worth RG. Cancer and thrombosis: the platelet perspective. Front Cell Dev Biol. 2017;4:147. PMID: 28105409; PMCID: PMC5214375
7. Günay Uçmak Z, Güvenç K. Malign meme tümörlü dişi köpeklerde klinik ve bazi hematolojik parametreler arasındaki ilişkinin değerlendirilmesi [Evaluation of relationship between clinical and some hematological parameters in bitches with malignant mammary tumor]. Turkiye Klinikleri J Vet Sci. 2019;10(2):45-52. doi: 10.5336/vetsci.2019-71596
8. Shevde LA, Samant RS. Role of osteopontin in the pathophysiology of cancer. Matrix Biol. 2014;37:131-41. PMID: 24657887; PMCID: PMC5916777

9. Weber GF. The cancer biomarker osteopontin: combination with other markers. Cancer Genomics Proteomics. 2011;8(6):263-88. PMID: 22086896
10. Wei R, Wong JPC, Kwok HF. Osteopontin -- a promising biomarker for cancer therapy. J Cancer. 2017;8(12):2173-83. PMID: 28819419; PMCID: PMC5560134
11. Bellahcène A, Castronovo V. Increased expression of osteonectin and osteopontin, two bone matrix proteins, in human breast cancer. Am J Pathol. 1995;146(1):95-100. PMID: 7856741; PMCID: PMC1870781
12. Allan AL, George R, Vantyghem SA, Lee MW, Hodgson NC, Engel CJ, et al. Role of the integrin-binding protein osteopontin in lymphatic metastasis of breast cancer. Am J Pathol. 2006;169(1):233-46. PMID: 16816376; PMCID: PMC1698777
13. Kaszak I, Ruszczak A, Kanafa S, Kacprzak K, Król M, Jurka P. Current biomarkers of canine mammary tumors. Acta Vet Scand. 2018;60(1):66. PMID: 30373614; PMCID: PMC6206704
14. Moschetta MG, Maschio LB, Jardim-Perassi BV, Gelaleti GB, Lopes JR, Leonel C, et al. Prognostic value of vascular endothelial growth factor and hypoxia-inducible factor 1α in canine malignant mammary tumors. Oncol Rep. 2015;33(5):2345-53. PMID: 25779537
15. Raposo-Ferreira TM, Salvador RC, Terra EM, Ferreira JH, Vechetti-Junior IJ, Tinucci-Costa M, et al. Evaluation of vascular endothelial growth factor gene and protein expression in canine metastatic mammary carcinomas. Microsc Res Tech. 2016;79(11):1097-104. PMID: 27543935
16. Queiroga FL, Pires I, Parente M, Gregório H, Lopes CS. COX-2 over-expression correlates with VEGF and tumour angiogenesis in canine mammary cancer. Vet J. 2011;189(1):77-82. PMID: 20675158
17. Ramchandani D, Weber GF. Interactions between osteopontin and vascular endothelial growth factor: implications for cancer. Biochim Biophys Acta. 2015;1855(2):202-22. PMID: 25732057
18. Yıldırım F, Sönmez K, Özyoğurtçu H, Şennazlı G, Gürel A, Gündüz MC, et al. Evaluation of Bcl-2, Bcl-XL and Bax expression and apoptotic index in canine mammary tumours. Kafkas Univ Vet Fak Derg. 2014;20(4):513-20. https://vetdergikafkas.org/uploads/pdf/pdf_KVFD_1625.pdf
19. Goldschmidt M, Peña L, Rasotto R, Zappulli V. Classification and grading of canine mammary tumors. Vet Pathol. 2011;48(1):117-31. PMID: 21266722
20. Owen LN. Clinical stages (TNM) of canine mammary tumors. TNM Classification of Tumors in Domestic Animals. 1st ed. Switzerland: World Health Organization; 1980. p.16-8.
21. Gupta K, Sood NK, Uppal SK, Mohindroo J, Mahajan S, Raghuath M, et al. Epidemiological studies on canine mammary tumour and its relevance for breast cancer studies. IOSR Journal of Pharmacy. 2012;2(2):322-33. https://iosrphr.org/papers/v2i2/ZJ022322333.pdf
22. Ben-Baruch A. Breast cancer progression: a "vicious cycle" of pro-malignancy activities is mediated by inflammatory cells, chemokines and cytokines. In: Meadows GG, ed. Integration/Interaction of Oncologic Growth. Cancer Growth and Progression. 1st ed. Netherlands: Springer; 2005. p.189-217.
23. Carvalho MI, Silva-Carvalho R, Pires I, Prada J, Bianchini R, Jensen-Jarolim E, et al. A comparative approach of tumor-associated inflammation in mammary cancer between humans and dogs. Biomed Res Int. 2016;2016:4917387. PMID: 28053982; PMCID: PMC5178344
24. Yamagami T, Kobayashi T, Takahashi K, Sugiyama M. Prognosis for canine malignant mammary tumors based on TNM and histologic classification. J Vet Med Sci. 1996;58(11):1079-83. PMID: 8959655
25. Tecles F, Caldín M, Zanella A, Membiela F, Tvarijonaviciute A, Subiela SM, et al. Serum acute phase protein concentrations in female dogs with mammary tumors. J Vet Diagn Invest. 2009;21(2):214-9. PMID: 19286500
26. Sharon Y, Raz Y, Cohen N, Ben-Shmuel A, Schwartz H, Geiger T, et al. Tumor-derived osteopontin reprograms normal mammary fibroblasts to promote inflammation and tumor growth in breast cancer. Cancer Res. 2015;75(6):963-73. PMID: 25600648
27. Weber GF, Lett GS, Haubein NC. Osteopontin is a marker for cancer aggressiveness and patient survival. Br J Cancer. 2010;103(6):861-9. PMID: 20823889; PMCID: PMC2966627
28. Saad ES, Milley KM, Al-Khan AA, Nimmo JS, Bacci B, Tayebi M, et al. Canine mixed mammary tumour as a model for human breast cancer with osseous metaplasia. J Comp Pathol. 2017;156(4):352-65. PMID: 28449818
29. Ibrahim T, Leong I, Sanchez-Sweatman O, Khokha R, Sodek J, Tenenbaum HC, et al. Expression of bone sialoprotein and osteopontin in breast cancer bone metastases. Clin Exp Metastasis. 2000;18(3):253-60. PMID: 11315099
30. Restucci B, Papparella S, Maiolino P, De Vico G. Expression of vascular endothelial growth factor in canine mammary tumors. Vet Pathol. 2002;39(4):488-93. PMID: 12126152
31. Santos AA, Oliveira JT, Lopes CC, Amorim IF, Vicente CM, Gärtner FR, et al. Immunohistochemical expression of vascular endothelial growth factor in canine mammary tumours. J Comp Pathol. 2010;143(4):268-75. PMID: 20570280
32. Anadol E, Yar Saglam AS, Gultiken N, Karakas K, Alcigir E, Alkan H, et al. Expression of iNOS, COX-2 and VEGF in canine mammary tumours and non-neoplastic mammary glands: association with clinicopathological features and tumour grade. Acta Vet Hung. 2017;65(3):382-93. PMID: 28956485
33. Wai PY, Kuo PC. Osteopontin: regulation in tumor metastasis. Cancer Metastasis Rev. 2008;27(1):103-18. PMID: 18049863
34. Pang H, Lu H, Song H, Meng Q, Zhao Y, Liu N, et al. Prognostic values of osteopontin-c, E-cadherin and β-catenin in breast cancer. Cancer Epidemiol. 2013;37(6):985-92. PMID: 24012693