JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Hematological and blood biochemical differences in dogs with mammary dysplasia/ hyperplasia, bening or malignant mammary tumors

Orkun Hacıoğlu[1], Rabia Buse Aksu[1], Sinem Yaren Akgül[1], Tuğba Seval Fatma Toydemir Karabulut[2]

1 Istanbul University-Cerrahpaşa, Institute of Graduate Studies, Istanbul, Turkey. 2. Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey. Hacıoğlu, O. ORCID: 0000-0003-4307-4897, Aksu, R. B. ORCID: 0000-0002-2572-4289, Akgül, S.Y. ORCID: 0009-0005-3777-2738, Toydemir Karabulut, T. S. F. ORCID: 0000-0001-6416-8337

Research Article

Volume: 8, Issue: 3
December, 2024
Pages: 273-281

ABSTRACT

Mammary tumors are the most common neoplasms in female dogs. There are many factors involved in the development of mammary tumors. Recently, there is an increasing need for cost-effective prognostic markers derived from hematological parameters in dogs and cats with cancer. Our study was designed as a retrospective study to determine the significance of hematologic parameters in female dogs bearing with mammary tumors. For this, hematocrit (HCT), blood biochemistry, and some clinical and histopathological findings of female dogs with mammary gland masses that had undergone mastectomy (n = 100) were included in our study. Mammary masses in female dogs are divided into five histopathological groups: Group 1 (malignant epithelial tumors), Group 2 (malignant mesenchymal tumors), Group 3 (malignant mixed tumors), Group 4 (benign tumors) and Group 5 (hyperplasia/dysplasia). The following hematologic parameters were evaluated: leukocytes (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BAS), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT),mean platelet volume (MPV), and neutrophil to lymphocyte ratio (NLR). Biochemical analysis included blood urea nitrogen (BUN), creatinine (CRE), alanine aminotransferase (ALT), glucose (GLU), total protein (TP), alkaline phosphatase (ALP), and albumin/globulin ratio (AGR). Comparisons were made between the groups according to histopathological tumor types. The most common histopathological type after mastectomy is malignant epithelial tumors (72%). For TP, a difference was found between G1 and G5 ($p<0.05$). For MON, a difference was found between G1 and G5, between G2 and G5, between G3 and G5, between G1 and G4, between G3 and G4 ($p<0.05$). For PLT, a difference was found between G2-G3 and G3-G4 ($p<0.05$). Although NLR was different between the groups and gets higher values with malignancy no statistical significance was found between the groups ($p>0.05$). While our study revealed some potential associations between hematologic parameters and histopathological tumor types, further studies with a larger and more diverse canine population are needed before these parameters can be reliably used as prognostic markers in female dogs with mammary masses.

Article History

Received: 30.05.2024
Accepted: 07.09.2024
Available online: 30.12.2024

Keywords: canine, mammary tumor, cbc, neutrophil-lymphocyte ratio, albumin-globulin ratio

DOI: https://doi.org/10.30704/http-www-jivs-net. 1475777

To cite this article: Hacıoğlu, O., Aksu, R. B., Akgül, S. Y., Toydemir Karabulut, T. S. F. (2024). Hematological and blood biochemical differences in dogs with mammary dysplasia/ hyperplasia, bening or malignant mammary tumors. ***Journal of Istanbul Veterinary Sciences, 8*(3), 247-281. Abbreviated Title*: J. İstanbul vet. sci.**

Introduction

Mammary tumors are the most common neoplasms (%50 of all tumors) in female dogs (Ariyarathna et al., 2020). While 80-90% of mammary tumors in cats are malignant, the malignancy in dogs is 50% (Kırşan and Canooğlu, 2016). Most of the mammary tumors in female dogs are of epithelial origin (Namagerdi et al., 2020). Female dogs with spontaneous mammary tumors have a variety of epidemiological, clinical, biological and genetic characteristics similar to those of women. For this reason, some researchers

*Corresponding Author: Orkun Hacıoğlu
E mail: haciogluorkun@gmail.com

recommend using these canine mammary tumors as models for comparative studies with humans (Estrela-Lima et al., 2010). The development of mammary tumors in female dogs is influenced by a complex interplay of factors, including breed predisposition, dietary habits, advanced age, environmental influences, neutering status, pseudo pregnancies and radiation exposure (Marquardt, 2003). The incidence of mammary tumors in female dogs increases significantly with age, while benign tumors can also occur at a younger age. The incidence of mammary tumors in female dogs is significantly influenced by the time of spaying. In prepubertal spayed bitches, the risk is remarkably low at 0.5%. However, the risk increases substantially to 8% for dogs spayed after their first estrus cycle and further rises to 26% for those spayed after their second estrus cycle (Egenvall et al., 2005). Although rare, mammary tumors can also occur in male dogs (Kırşan and Canooğlu, 2016). While the incidence of mammary tumor is generally higher in small breeds than in large breeds, certain large dog breeds also have a significant risk (Sorenmo et al., 2013). Several purebred dog breeds have a significantly higher predisposition to mammary tumors compared to other breeds. These breeds include the English Springer Spaniel, the Cocker Spaniel, English Setter, Pointer, German Shepherd, Maltese, Yorkshire Terrier, Dachshund, Puli, Toy Poodle and Miniature Poodle (Moe L, 2001).

There is a growing need for the identification of cost-effective prognostic markers derived from hematologic parameters in dogs and cats with neoplastic or inflammatory diseases. Cancer-related anemia, a common complication observed in patients with various types of tumors, can adversely affect prognosis and response to treatment. In addition to cancer-related anemia, a range of biochemical abnormalities have been found in oncology patients, including hypercalcemia, hypoglycemia, hypoproteinemia, and elevated levels of total alkaline phosphatase and the corresponding isoenzymes. A study by Oliveira et al. (2022), which examined female dogs with mammary tumors, found that the most common hematological and biochemical changes were normocytic normochromic anemia, neutrophilic leukocytosis, monocytosis, elevated ALT and AST and hypoalbuminemia. A comparative analysis of hematological parameters in dogs with benign and malignant mammary tumors revealed that anemia was the most significant abnormality in the benign group, while the malignant tumors were characterized by leukopenia, predominantly neutropenia, thrombocytosis and hyperproteinemia (Lallo M.A et al., 2016). Red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), determined as part of routine blood count analysis, have been shown to be significant biomarkers related to systemic inflammation and cancer biology, providing valuable insights for cancer detection, progression and survival prognosis (Divsalar et al., 2021). Another study conducted in dogs with lymphoma, osteosarcoma, mast cell tumors and soft tissue sarcoma has shown that WBC and NLR provide prognostic information (Petrucci et al., 2021). In dogs with mast cell tumors and soft tissue sarcomas, higher NLR was associated with more aggressive tumors. In cats with injection-site sarcomas, WBC and NLR have been shown to be prognostic factors for local recurrence (Petrucci et al., 2021). In a study on dogs, it was found that a high pre-treatment NLR value (NLR > 5) was associated with a lower survival rate. However, AGR (Albumin/ Globulin Rate) was not shown to have a significant impact on tumor malignancy and it is hypothesized that NLR could be used as a prognostic marker for disease severity (Uribe Querol et al., 2023). Breast tumors that are Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor 2 (Her-2) negative are referred to as Triple Negative Breast Cancers (TNBC), and a high rate of NLR has been shown to be associated with recurrence and poor prognosis (Petrucci et al. al., 2021). The prognostic utility of routinely determined hematologic and biochemical parameters in oncologic patients in veterinary medicine remains poorly understood. The aim of our study is investigate the presence of significant differences in clinically assessed hematologic and biochemical serum parameters in female dogs with malignant mammary tumors, benign mammary tumors and hyperplasia/dysplasia.

Materials and Method

Patients

Female dogs with mammary masses (n = 100) aged between 3-17 years, who applied to Istanbul University -Cerrahpaşa Faculty of Veterinary Medicine, Obstetrics and Gynecology Clinic between 2018 and 2022, were included in our study. Our retrospective case study includes the comparison of blood tests performed before mastectomy in female dogs with the histopathological results of mammary tissue taken after mastectomy. The female dogs were divided into five groups based on their mammary gland pathology (Goldschmidt et al., 2011): female dogs with malignant epithelial tumors (Group 1, n = 72), female dogs with malignant mesenchymal tumors (Group 2, n = 9), female dogs with malignant mixed tumors (Group 3, n =

5), female dogs with benign tumors (Group 4, n = 7) and female dogs with hyperplasia/dysplasia (Group 5, n = 7). The breed distribution of the female dogs included in the study was as follows: mixed breeds (n=20), Cocker Spaniel (n=18), Terrier (n=18), Golden Retriever (n=14), Yorkshire Terrier (n=5), Pinscher (n=3), Cavalier King Charles Spaniel (n=3), Beagle (n=3), Russian Fino (n=2), Pekingese (n=2), Boxer (n=2), German Shepherd Dog (n=2), Setter (n=1), Rottweiler (n=1), Labrador Retriever (n=1), Sivas Kangal (n=1), Jack Russell Terrier (n=1), French Bulldog (n=1), Chow Chow (n=1) and Akita Inu (n=1). The mean age and neuter status for each group are shown in Table 1. Written or verbal informed consent was obtained from dog owners for the use of their dogs' blood and tissue results. As our study is a retrospective study, ethics committee approval was not required.

Blood Samples

Blood samples are routinely taken from female dogs that are brought to our hospital with the complaint of a mass in the mammary gland and are subsequently scheduled for mastectomy. For this purpose, blood is taken from the V. cephalica antebrachii into EDTA-containing tubes for the hemogram and into heparin-containing tubes for the biochemical analysis of the serum. Blood tests were performed in the central laboratory of the university. The data of the female dogs included in our retrospective study were obtained from the laboratory's information system. For complete blood count, the results obtained from the hemogram device (Procyte Dx Hematology Analyzer, Idexx, USA) and biochemistry device (DRI-CHEM NX600, Fujifilm, Japan) in the laboratory were taken from the laboratory information system and used. The following parameters in the hemogram results were included in the study: leukocyte (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MON), eosinophil (EOS), basophil (BAS), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT) and mean platelet volume (MPV). In the biochemistry results, the following parameters were included in the study: Blood urea nitrogen (BUN), creatinine (CRE), alanine aminotransferase (ALT), glucose (Glu), total protein (TP), albumin (ALB) and alkaline phosphatase (ALP). To high GLU levels were excluded from the groups for the correct statistical results of ANOVA. The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the total number of neutrophil by the total number of lymphocyte. The albumin-to-globulin ratio (AGR) was calculated by dividing the albumin concentration by the globulin concentration (Uribe Querol et al., 2023).

Histopathology

The histopathology results of the female dogs included in our retrospective study were obtained from the hospital's automation system and used. Routinely, mammary tissue examinations of dogs that have undergone mastectomy in our faculty are carried out in the laboratory of our university's Department of Pathology. Briefly, for histopathology, mammary tissue samples taken from mastectomies are fixed in 10% buffered formalin solution for 24 hours and embedded in paraffin blocks. Embedded tissue and cell block samples were processed using routine tissue processing procedures, and 5-µm-thick serial sections were prepared from all samples using a rotary microtome. Section samples from each mammary tissue and lymph node sample are stained with hematoxylin-eosin (H&E) and evaluated under a light microscope. As a result of examining the tissues stained with H&E under a light microscope, the mammary gland tissues of the bitch were histopathologically classified into five groups (Figure 1). In female dogs with multiple mammary tumors, all comparisons were based on histopathological result of primary tumor.

Statistical Analysis

All data analyses were performed using IBM SPSS version 27.0. The categorical and continuous variables were expressed using descriptive statistics (mean±standard deviation). In addition, the Levene's test was used to check the homogeneity of variance, a prerequisite for parametric tests. The normality assumption was verified using the Kolmogorov-Smirnov and Shapiro-Wilk test. The groups (group 1-5) were added to the statistical model as between-

Table 1. Mean age and sterilization situations of the groups.

Variables	G1	G2	G3	G4	G5	Total
N	72	9	5	7	7	100
Age (Mean ± SD)	10.35 ±2.87	10.55 ±3.43	10.6 ±0.48	8.92 ±2.17	7.21 ± 2.74	10.06 ±2.93
OVH +	16 (22.22%)	2 (22.22 %)	0 (0 %)	3 (42.85 %)	1 (14.28 %)	22 (22 %)
OVH -	56 (77.78%)	7 (77.78 %)	5 (100 %)	4 (57.15 %)	6 (85.72 %)	78 (78 %)

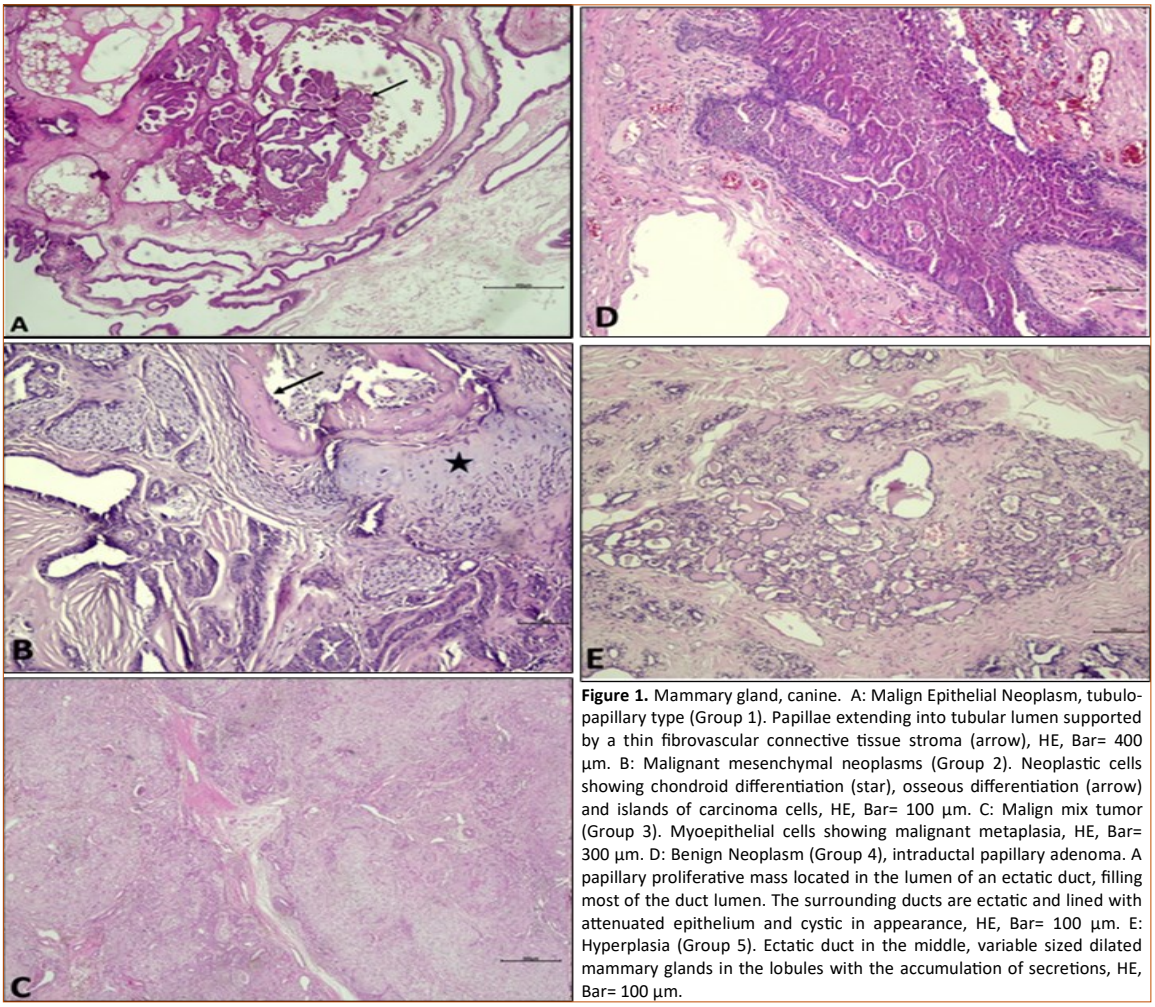

Figure 1. Mammary gland, canine. A: Malign Epithelial Neoplasm, tubulo-papillary type (Group 1). Papillae extending into tubular lumen supported by a thin fibrovascular connective tissue stroma (arrow), HE, Bar= 400 µm. B: Malignant mesenchymal neoplasms (Group 2). Neoplastic cells showing chondroid differentiation (star), osseous differentiation (arrow) and islands of carcinoma cells, HE, Bar= 100 µm. C: Malign mix tumor (Group 3). Myoepithelial cells showing malignant metaplasia, HE, Bar= 300 µm. D: Benign Neoplasm (Group 4), intraductal papillary adenoma. A papillary proliferative mass located in the lumen of an ectatic duct, filling most of the duct lumen. The surrounding ducts are ectatic and lined with attenuated epithelium and cystic in appearance, HE, Bar= 100 µm. E: Hyperplasia (Group 5). Ectatic duct in the middle, variable sized dilated mammary glands in the lobules with the accumulation of secretions, HE, Bar= 100 µm.

-subjects effects, and a one-way ANOVA and Tukey's HSD (as a post hoc test) tests was performed to compare mammary tumor disease on each hematological parameter between the groups. Additionally, the Kruskal-Wallis test was used in cases where the assumptions were not valid. A value of $p < 0.05$ was used to indicate statistical significance.

Results

Data on hematologic and blood biochemistry results for each group are shown in Table 2 and Table 3, respectively. An increased mean MON count was observed in Group 2 (Table 2). MCV value was below the normal reference range in Group 2 (Table 2). PLT was above the normal reference range in Group 3 (Table 2). In addition to the average hematology values, the WBC count was found to be above the reference range in ten female dogs in Group 1 and in three female dogs in Group 2. Despite an increased mean MON value in Group 2, MON values above the reference range were found in 21 female dogs in Group 1. LYM levels were above the normal reference range in only two female dogs in Group 1. While the mean NEU value was within the reference range in all groups, NEU values above the reference range were found in 19 female dogs in Group 1 and in two female dogs each in Group 2 and Group 3. Although the mean RBC value was within the reference range in all groups, nine female dogs in Group 1 and two female dogs in Group 2 had RBC values below the normal reference range. While mean HGB and HCT values were within the reference range in all groups, HGB values were found to be below the normal reference range in nine female dogs in Group 1 and four female dogs in Group 2. Similarly, HCT levels were below the normal reference range in 12 female dogs in Group 1 and five female dogs in Group 2. Despite an decreased mean MCV value in Group 2, 15 female dogs in Group 1, three female dogs in Group 3, a female dog in Group 4 and 2 female dogs in Group 5 had MCV values below the normal reference range. While the mean PLT value was elevated in Group 3, 12 female dogs in Group 1, two female dogs in Group 2 and a female dog each in Group 4 and Group 5 had PLT values above the normal reference range. While the mean values for all biochemical variables in the blood were within the normal reference range in all groups, the BUN and CRE levels were below the normal reference range in ten female dogs in Group 1. ALT levels were above the normal reference range in six female dogs in Group 1 and a female dog in Group 5. ALP levels were above the normal reference range in six female dogs in Group 1, two female dogs in Group 2 and a female dog in Group 3. The TP levels were above the normal reference range in eight female dogs in Group 1. GLU levels were below the normal reference range in a female dog in Group 1 and above the normal reference range in a female dog in Group 2.

Table 2. Data of the hemogram variables and NLR (Neu/ Lym) values of the groups.

Parameters	G1	G2	G3	G4	G5	Reference Ranges	P
WBC (K/µL)	13.37 ±11.52	15.64 ±8.36	12.62 ±2.95	8.79 ±3.46	9.18 ±1.87	5.05-16.76	0.126^{K}
Lym (K/µL)	2.22 ±1.53	2.18 ±0.99 ^{b}	2.22 ±0.25	1.56 ±0.48	1.90 ±0.66	1.05-5.10	0.296^{K}
Neu (K/µL)	9.61 ±9.59	11.48 ±6.45	8.32 ±2.11	6.22 ±2.76	6.00 ±1.48	2.95 - 11.64	0.191^{K}
Mon (K/µL)	1.07 ±0.74	1.40 ±1.46	1.05 ±0.28	0.59 ±0.29	0.59 ±0.15	0.16 - 1.12	**0.025**^{K}
Bas (K/µL)	0.02 ± 0.02^{a}	0.02 ± 0.02^{a}	0.00 ± 0.00^{a}	0.02 ± 0.01^{a}	0.07 ±0.11	0.00 - 0.10	0.368^{K}
Eos (K/µL)	0.42 ±0.28	0.54 ±0.52	0.84 ±0.60	0.39 ±0.22	0.60 ±0.33	0.06 - 1.23	0.299^{K}
RBC (K/µL)	6.73 ±0.96	6.44 ±1.24	7.45 ±0.84	7.01 ±0.35	6.75 ±0.98	5.65 - 8.87	0.427^{A}
HGB (g/dL)	15.61 ±2.16	14.21 ±2.91	16.7 ± 2.03	16.24 ±1.04	15.57 ±1.60	13.1 - 20.5	0.256^{A}
HCT (%)	43.26 ±6.68	39.22 ±8.54	46.94 ±6.19	45.07 ±3.62	40.91 ±6.73	37.3 - 61.7	0.227^{A}
MCV (fL)	64.14 ±3.49	60.73 ±3.46	62.88 ±3.08	64.25 ±3.19	63.00 ±2.73	61.6 - 73.5	0.086^{A}
MCH (pg)	23.21 ±1.39	22.03 ±1.61	22.40 ±0.65	23.15 ±1.25	23.17 ±1.32	21.2 - 25.9	0.156^{A}
MCHC (g/dL)	36.18 ±1.56	36.30 ±2.12	35.64 ±0.86	36.08 ±1.26	36.78 ±0.76	32.0 - 37.9	0.796^{A}
RDW (%)	16.64 ±2.46	16.48 ±3.14	17.80 ±1.51	16.25 ±1.69	15.21 ±1.20	13.6 - 21.7	0.466^{A}
PLT (K/µL)	364 ±120.74 ^{a,b}	271 ±150.29 ^{a}	494 ±155.65 ^{b,A}	236 ±117.27 ^{a,b,B}	312.8 ±43.7 ^{a,b,A,B}	148 – 484	0.005^{A}
MPV (fL)	10.05 ±2.32	11.43 ±3.54	10.58 ±1.35	10.74 ±1.37	9.41 ±1.50	8.7 - 13.2	0.446^{A}
NLR (Neu /Lym)	4.48 ± 2.84	5.80 ±3.38	3.74 ±0.86	4.05 ±1.15	3.43 ±1.24		0.500^{K}

BAS: Basophil; EOS: Eosinophil; HCT: Hematocrit; HGB: Hemoglobin; LYM: Lymphocyte; MCH: Average Amount of Erythrocyte Hemoglobin; MCHC: Mean Erythrocyte Hemoglobin Concentration; MCV: Mean Erythrocyte Volume; MON: Monocyte; MPV: Average Platelet Volume; NEU: Neutrophil; NLR: Neutrophil-to-Lymphocyte Ratio; PLT: Platelet; RBC: Erythrocyte; RDW: Erythrocyte Distribution Width; WBC: White Blood Cells; a,b,A,B: Different superscript letters on the same row indicate statistically significant difference (P<0.05). ANOVA, K: KRUSKAL-WALLIS

The one-way ANOVA revealed that there was a statistically significant difference in the mean value of TP levels between G1 and G5 (p<0.05). For PLT, a difference was found between G2-G3 and G3-G4 (p<0.05). Kruskal-Wallis revealed that there was a statistically significant difference in the test mean values of MON levels between G1-G5, G2-G5, G3-G5, G1-G4 and G3-G4 (p<0.05). Although NLR was different between the groups and gets higher values with malignancy no statistical significance was found between the groups (p>0.05).

The mastectomy revealed that malignant epithelial tumours were the most common histopathological type (72%) among the histopathological findings obtained. The most common histopathological subtype was complex carcinoma (13%), followed by tubular carcinoma (8%) and tubulopapillary carcinoma (7%). Table 4 shows the types of mammary tumors found in the dog breeds included in the study.

Table 3. Data of the biochemical variables of the blood belonging to the groups.

Parameters	G1	G2	G3	G4	G5	Reference	P
ALB (g/dL)	3.29 ±1.24	2.811 ±0.37	3.26 ±0.38	3.14 ±0.25	3.27 ±0.27	2.2 – 4.0	0.160^{K}
ALP (U/L)	83.63 ±53.61	72.57 ± 30.59	97.75 ±35.78	70.42 ±29.87	44.28 ±12.88	23 – 212	0.121^{K}
ALT (U/L)	60.77 ±40.77	42.44 ±20.65	68 ±8.62	53.14 ±28.28	62.71 ±30.11	10–125	0.130^{K}
BUN (mg/dL)	13.82 ±5.62	17.84 ±10.44	13.80 ±6.24	15.01 ±2.77	15.15 ±5.62	7–27	0.548^{A}
CRE (mg/dL)	0.98 ±1.56	1 ±0.32	0.9 ±0.16	0.84 ±0.21	0.91 ±0.31	0.5-1.8	0.400^{K}
BUN/CRE	19.05 ±9.86	17.72 ±6.29	14.6 ±4.49	18.66 ±4.41	20.53 ±10.63		0.819^{K}
GLU (mg/dL)	103.76 ±11.76	104 ±11.19	100.6 ±11.25	101.42 ± 6.13	115.14 ±9.56	74–143	0.131^{A}
TP (mg/dL)	7.39 ±0.70 ^{a}	7.05 ±0.35 ^{a,b}	7.56 ± 0.34 ^{a,b}	6.92 ± 0.46 ^{a,b}	6.61 ±0.46 ^{b}	5.2-8.2	0.013^{A}
AGR	0.77 ± 0.20	0.70 ±0.13	0.78 ±0.20	0.85 ±0.15	1.07 ±0.23	≤ 1.0	0.056^{K}

AGR: Albumin-globulin ratio; ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; BUN: Blood Urea Nitrogen; CRE: Creatinine; GLU: Glucose; TP: Total Protein; a,b: Different or combinations of superscript letters on the same row indicate statistically significant difference (P<0.05). ANOVA, K: KRUSKAL-WALLIS

Table 4. Dog breeds and histopathological subtypes distribution of the groups.

Dog Breeds	Histopathologic subtypes and number of patients (n)
German Shepherd Dog	Carcinosarcoma (n=1), Malignant Mixed Tumor (n=1)
Akita Inu	Carcinosarcoma (n=1)
Beagle	Tubular Carcinoma (n=1), Carcinoma (n=1), Malignant Mixed Tumor (n=1)
Boxer	Adenosquamos Carcinoma (n=1), Carcinoma Mixed Type (n=1)
Chow Chow	Carcinoma (n=1)
Cocker Spaniel	Adenocarcinoma (n=3), Adenosis (n=2), Basic Papillary Adenocarcinoma (n=1), Duct Ectasia and Adenozis (n=1), Fibroadenomatous Change (n=1), Carcinoma Complex Type (n=2), Malignant Mixed Tumor (n=1), Sarcomas (n=1), Solid Adenocarcinoma (n=2), Solid Carcinoma (n=1), Tubular Carcinoma (n=2), Tubulopapillary Carcinoma (n=1)
French Bulldog	Tubular Adenocarcinoma (n=1)
Golden Retriever	Tubulopapillary Carcinoma (n=3), Tubulopapillary Adenocarcinoma (n=2), Malignant Myoepithelium (n=2), Malignant Mixed Tumor (n=1), Complex Carcinoma (n=2), Carcinosarcoma (n=1), Carcinoma Mixed Type (n=1), Intraductal Xanthomatous Fibrous Breeding (n=1), Ductal Carcinoma (n=1)
Jack Russel Terrier	Benign Mixed Tumor (n=1)
King Charles	Solid Adenocarcinoma (n=1), Carcinosarcoma (n=1), Adenoma (n=1),
Sivas Kangal	Complex Carcinoma (n=1)
Labrador Retriever	Tubular Carcinoma (n=1)
Mixed Breed	Tubular Carcinoma (n=1), Tubulopapillary Carcinoma (n=1), Solid Adenocarcinoma (n=1), Tubular Adenocarcinoma (n=3), Complex Carcinoma (n=2), Tubular Carcinoma- Mixed Type (n=1), Carcinoma (n=2), Adenocarcinoma (n=1), Complex Adenocarcinoma (n=1), Lipid-Rich Carcinoma (n=1), Squamous Cell Carcinoma (n=1), Carcinosarcoma (n=1), Osteosarcoma (n=1), Fibroadenoma (n=1), Chondroma (n=1), Fibroadenomatous Change (n=1)
Pekingese	Adenoma (n=1), Rhabolomyosarcoma (n=1)
Pinscher	İntraductal Papillary Adenoma (n=1), Tubulopapillary Carcinoma (n=1), Endocrinopathy (n=1)
Rottweiler	Tubular Carcinoma (n=1)
Russian Fino	Comedocarsinoma (n=1), Papillar Carcinoma (n=1)
Setter	Tubulopapillary Carcinoma (n=1)
Terrier	Benign Mixed Tumor (n=1), Ductal Adenocarcinoma (n=1), Hyperplasia (n=1), Carcinoma (n=1), Carcinoma Mixed Type (n=1), Carcinosarcoma (n=1), Complex Adenocarcinoma (n=1), Complex Carcinoma (n=1), Malignant Myoepithelioma (n=1), Tubular Adenocarcinoma (n=1), Tubular Carcinoma (n=1),
Yorkshire Terrier	Tubular Adenocarcinoma (n=1), Malignant Mixed Tumor (n=1), Complex Carcinoma (n=1), Intraductal Papillary Carcinoma (n=1), In Situ Carcinoma (n=1)

Discussion and Conclusion

In a study hematological examination of women with breast tumors, the mean values of WBC, RDW and MPV were higher but the mean values of RBC, HGB, HCT, MCV and MCH were lower than those of healthy women (Divsalar et al., 2021). A study of 30 dogs with advanced-stage tumor masses in the mammary glands showed a significant decrease in RBC, HGB and PCV values compared to healthy dogs. In addition, slight reductions in MCV and MCH were observed (Hasan S et al., 2015). A study comparing healthy female dogs and female dogs with mammary tumors showed a decrease in RBC, HGB and HCT levels in the mammary tumor group (Satilmis F et al., 2022). In our study, the MCV value is also below the mean group MCV value in Group 2. The study conducted by Divsalar et al. showed that the parameters RDW and MCV represent the best differential diagnostic hematological potential for mammary tumor in female dogs. In our study, although no statistically significant difference in WBC values was found between the groups, ten female dogs in Group 1 and three female dogs in Group 2 were found to have an increased WBC count. Consistent with previous studies, the mean WBC values were within the normal range in all groups. Divsalar et al. reported an increase in WBC count in the patients compared to the control group, but the average WBC remained within the normal reference range (Divsalar et al., 2021). A similar situation was found by Satilmis et al.in which there was no statistical difference in the WBC value between dogs with mammary tumors and healthy dogs, but it was found that the WBC value increased significantly when the size of the tumor increased and was seen in many mammary lobes (Satilmis F et al., 2022). In another study, a 1.5 to 3 fold increase in the WBC count was found in the examined group compared to healthy animals (Hasan et al., 2015). In a study conducted on 246 female dogs with mammary tumors, it was found that hematological abnormal changes were less pronounced in dogs with benign mammary tumors. In dogs with malignant mammary tumors, hematological abnormal changes were found in 55% of the samples examined, and it was found that there is a close

relationship between cancer progression and hematological changes. When the blood count of female dogs with malignant tumors is compared with that of female dogs with benign tumors and the healthy control group, thrombocytosis was found in 38%, hyperproteinemia in 34% and leukopenia with predominant neutropenia in 34% (Lallo M.A et al., 2016). In our study, neutropenia and leukopenia were not observed in any groups. But increased mean PLT count was observed in Group 3. Additionally, 12 dogs in Group 1, two dogs in Group 2 and a dog in Group 4 and Group 5 were found to have a value above the normal reference range. Our statistical analysis showed that the PLT value was significant differences between G2-G3 and G3-G4 ($p<0.05$). PLT values can be used as a predictor for different types of cancer in humans (Jurasz P. et al., 2004). Breast cancer patients with elevated PLT levels tend to have poorer survival (Taucher S. et al., 2003). A study of hematologic parameters in dogs with different types of cancer showed no significant differences in PLT counts among the different cancer groups (Andreasen E.B. et al., 2012). Another study found that PLT levels in female dogs with mammary carcinoma were 3.3% above the normal range (Stockhaus C. et al., 1999). In another study, it was observed that the increased TP was observed in female dogs with mammary tumors (Satilmis F et al., 2022). A study of dogs with mammary gland neoplasms showed a statistically significant decrease in the mean MON count compared to a group of healthy control dogs (Gangwar K. et al., 2024). In contrast to our results, in a study investigating tumor progression in 43 female dogs with mammary tumors, an increase in MON levels was observed in dogs with inflammatory carcinomas (Oliveira M.R. et al., 2022). In our study, a significant difference in mean monocyte counts was found between different groups. These results suggest that monocytes could serve as a potential biomarker to differentiate between malignant and benign/hyperplastic tumors, but further studies are needed to support this hypothesis.

A study in which the values of BUN, CRE, ALT and AST were compared in 30 dogs with mammary tumors and 10 healthy dogs showed no significant difference between these values in the two groups (Srikanth N., et al 2021). In a separate study of 30 dogs with advanced-stage mammary tumors, the values for BUN, CRE, ALT and AST were within the normal range (Hasan et al, 2015). In our study, 100 female dogs complaining of mammary tumors were grouped according to their histopathological subtypes. The mean BUN, CRE and ALT values were within the normal reference ranges in all groups, which is consistent with the results of this study. In another study based on previous research, ALT enzyme activity was found to decrease in dogs with malignant mammary tumors (Satilmis F et al., 2022), but in our study, we only found an increase in ALT enzyme in six female dogs in Group 1, and in all groups, the mean value was within the normal reference range. Therefore, further studies are needed to establish a relationship between mammary tumor and ALT enzyme level. In a study on dogs with mammary tumors, a non-significant increase in TP values was observed in a few patients (Nandhini S. et al., 2022). In our study, although the mean TP value was within the normal reference range in all groups, there was a statistically significant difference between G1 and G5 ($P<0.05$).

Recent studies have found consistent results between the diabetes and the risk of pancreatic, liver, endometrial and colon/rectal cancer in humans. Data on esophageal, stomach, prostate and breast cancer are more limited and conflicting. In a nearly 10-year study examining the association between fasting serum glucose levels and cancer in Korean women and men between the ages of 30 and 95, the mortality rate for women with breast cancer was 6.0 for those with fasting glucose levels below 90, 7.5 for those with fasting glucose levels of 90-109, and 5.2 for those with fasting glucose levels of 110-125, while the rate for those between 126-139 and 140 and above 140 was 6.1, the rate for those with diabetes was 9.8. The breast cancer incidence rate for women is 60.2 for fasting glucose levels below 90, 63.8 for fasting glucose levels 90-109, 68.7 for levels 110-125, 65.2 for fasting glucose levels 126-139, 55.4 for fasting glucose levels 140 and above 140, and 76.9 for those with diabetes. Hyperinsulinemia is considered a possible risk factor for breast cancer, which is confirmed by laboratory findings. In that study, fasting blood glucose levels were found to increase the risk of breast cancer in women (Jee, S et all., 2005). In our study, no statistically significant difference was found in mean GLU values between the groups. Further studies with more dogs are needed to determine the prognostic relevance of GLU level and mammary tumour development.

Inflammation is part of the tumor microenvironment and influences every step of tumorigenesis, cellular and biochemical blood markers of systemic inflammation, such as neutrophil-to-lymphocyte ratio (NLR) and albumin-to-globulin ratio (AGR), have been proposed as prognostic factors for cancer development in humans. The NLR was calculated by dividing the total number of neutrophils by the total number of lymphocytes. The AGR was calculated by dividing the albumin concentration to the globulin concentration. Although NLR and AGR levels

have not been adequately studied as prognostic factors for cancer development in veterinary medicine, 45 dogs with mammary tumors and 25 healthy dogs were used in a study investigating the relationship between NLR and AGR levels and mammary tumors in female dogs (Uribe Querol et al., 2023). A comparison between the healthy, benign and malignant mammary tumor groups, the NLR values were found to be statistically different ($p<0.05$). The AGR value was calculated preoperatively, and similar between the healthy and female dogs with mammary tumor. According to those results, the AGR value alone could not provide a prognostic value for mammary tumors in female dogs (Uribe Querol et al., 2023). Additionally, the NLR value is not a sufficient indicator for determining tumor type. The NLR value could become a simple and cost-effective tool to help make therapeutic decisions from the outset, as an accurate diagnosis often requires a more invasive and expensive procedure (Uribe Querol et al., 2023). In veterinary medicine, AGR varies in bacterial infections in cats and parasitic infections in dogs (Uribe Querol et al., 2023). The combination of NLR and AGR is reported to have better predictive value in patients with triple-negative breast cancer (Uribe Querol et al., 2023). In our study, the mean AGR value was lower in female dogs with mammary tumor than in female dogs with mammary hyperplasia/ dysplasia, but no statistically significant difference was found for the AGR value between the groups. It was observed that NLR was highest in Group 2 and lowest in Group 5. No statistical significance was found for the NLR value between the groups ($p>0.05$).

In the study by Lallo et al. the average age of female dogs with mammary tumors was 10.5 ± 3.7 years and the most common breed was mixed breed with 48%, Poodles with 29%, Rottweilers with 6% and the rest were various other breeds. In our study, the mean age was 10.06 ± 2.93 years and the most common breed was mixed breed at 20%, followed by Cocker Spaniel at 18%, Terrier at 18% and the rest were various other breeds. As can be seen in Table 1, the average age of Groups 1-2-3 is higher than that of Groups 4-5, and it was seen that the average age of dogs with malignant mammary tumors is higher than that of dogs with benign mammary tumors which is a similar result of the study by Uribe-Querol et al., 2023. In another study conducted on 12 dogs with mammary tumors, the average age was 8.42 ± 1.88 years (Satilmis F et al., 2022).

In another study conducted on 51 female dogs with mammary tumors, 31 dogs were found to have benign mixed tumors and 20 dogs were found to have carcinomas. When dogs with carcinomas are divided into subtypes, the most common are four Papillary Carcinomas, four Solid Carcinomas, three Tubulopapillary Carcinomas and three Tubular Carcinomas (Estrela Lima, 2010). In our study of 100 dogs, in contrast to this study, the most common histopathological type was a malignant epithelial tumor (72%). The most common histopathological subtype was complex carcinoma (13%), followed by tubular carcinoma (8%) and tubulopapillary carcinoma (7%), respectively.

In conclusion, in this study the hemogram and biochemical analysis of female dogs (n=100) complaining of a mass in the mammary glands were examined and compared according to their histopathological types. Further studies in more animals are needed to use the differences in hemogram and biochemical values as predictive markers in patients complaining of mammary tumors/masses. According to the results of our study, it will be possible to make a prognosis based on the blood test results during the examination of the patient in the clinical setting. Our study is a guide for future studies.

Acknowledgements:

The authors thanks Prof. Dr. Bilge Acar Bolat for the help with the statistical analysis.

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