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The Effect of Size and Clinical Staging of Mammary Tumors on Blood Parameters in Bitches

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ABSTRACT

***Background*:** Mammary tumors are the most common type of tumor in female dogs and account for 50% of all tumors in dogs. The clinical prognosis of canine mammary tumors is strongly affected by the size, stages, histological type, and grade of tumor; mitotic index; and nearby and distant metastasis. In canine mammary tumors, it is recommended that prognostic evaluation should also include complete blood count, serum biochemistry, and blood gases in addition to tumor size and stage. This study aimed to investigate the effect of tumor size, volume, and clinical stage on complete blood count, blood gas analysis, and serum biochemical parameters in bitches with mammary tumors and the correlation between them.

***Materials, Methods & Results*:** The study included a total of 18 bitches of different breeds, aged 6-15 years, of which 12 had mammary tumors and 6 were healthy. Thoracic X-rays were performed on bitches with mammary tumors in ventrodorsal and laterolateral positions to evaluate lung metastasis. Blood samples were collected from the cephalic vein from bitches in both groups in 2 different tubes (with plastic gel and ethylenediaminetetraacetic acid), 5 mL each, to perform complete blood count and evaluate blood gases and serum biochemical parameters. Blood samples were collected from the animals at the time of initial examination without any intervention. Analysis of the blood showed that bitches with mammary tumors had decreased levels of RBC, HCT, HGB, potassium, TCO_2, base excess, THbc, and ALT enzyme activity and increased levels of lactate, total protein, cholesterol, triglyceride, LDL, uric acid, and ALP and LDH enzyme activities compared with those in the control group. Furthermore, the dogs with a primary tumor of > 5 cm were found to have significantly higher levels of WBC, lactate, total protein, triglyceride, LDL, uric acid, and ALP and LDH enzyme activities and significantly lower levels of RBC and THbc compared with those in the control group. Bitches with tumors in multiple mammary lobes were found to have significantly higher levels of WBC, total protein, triglyceride, LDL, and ALP and LDH enzyme activities and significantly lower levels of RBC, HCT, HGB, TCO_2, THbc, and ALT enzyme activity compared with those in the control group. Based on the laboratory findings and approval of the owners of the dogs, mammary tissues containing the tumor and lymph nodes were surgically removed. After the operation, the removed mammary tissues were evaluated for size and volume. Clinical staging of the tumors was performed based on the size of the primary tumor (T), nearby lymph nodes (N), and metastasis (M) in accordance with the criteria set by WHO. Clinical staging of the tumors was, thus, based on the tumor, nodes, and metastases (TNM) score obtained according to the following system: Stage I: $T_1N_0M_0$, Stage II: $T_2N_0M_0$, Stage III: $T_3N_0M_0$, Stage IV: $T_{any}N_1M_0$, Stage V: made as $T_{any}N_{any}M_1$.

***Discussion*:** Mammary tumors are the most common type of neoplasm in bitches and, thus, cause serious problems in veterinary medicine. Tumors are significantly correlated with better prognosis compared with larger tumors. Based on this finding, this study investigated the effect of size, volume, and stage of mammary tumors in bitches on some blood parameters and the correlation between them. Therefore, it was concluded that clinical staging and evaluation of blood parameters could be useful in the diagnosis, treatment, and prediction of prognosis in canine mammary tumors. This study found that bitches with mammary tumors exhibited significant changes in their blood parameters (complete blood count, blood gas analysis, and serum biochemistry). The results obtained from this study may contribute to the development of approaches to the diagnosis, prediction of prognosis, and treatment of canine mammary tumors.

Keywords: blood gas analysis, complete blood count, dogs, mammary tumor, serum biochemistry, tumor volume.

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INTRODUCTION

Mammary tumors are the most common type of tumor in female dogs and account for 50% of all tumors in dogs and 20% of skin tumors [22,32]. Although all bitches are likely to develop mammary tumors as they advance in age, intact bitches have higher incidence rates of mammary tumors than spayed bitches [40].

The clinical prognosis of canine mammary tumors is strongly affected by the size, stage, histological type, and grade of tumor; mitotic index; and nearby and distant metastasis [5,34,36]. Thus, the prognostic evaluation of mammary tumors uses criteria developed by the World Health Organization. Clinical staging of the tumor helps to predict the spread of tumors [5,36]. The clinical stage of mammary tumors is determined based on the size of the primary tumor (T), spread to nearby lymph nodes (N), and the presence of metastasis to distant tissues (M) [35].

In canine mammary tumors, it is recommended that prognostic evaluation should also include complete blood count, serum biochemistry, and blood gases in addition to tumor size and stage. Evaluating these parameters provides information to veterinarians in taking treatment decisions and planning the treatment because mammary tumors can cause significant changes in the general condition of the bitch and the blood parameters [36].

The present study attempted to evaluate whether a correlation can be established between tumor size, stage, and volume and serum biochemistry and complete blood count in clinical practice without histopathological classification.

MATERIALS AND METHODS

Animals

The study used a total of 18 female dogs of different breeds, which were > 5 kg and aged 6-15 years, of which 12 had mammary tumors and 6 were healthy (introduce to the clinic for genital examination).

Mammary tumors were diagnosed based on anamnesis, palpation, inspection, and clinical findings. Clinical examinations were performed to evaluate the characteristics, size, and location of the mass in the mammary gland as well as the enlargement of lymph nodes, if any. Thoracic radiography was performed to evaluate lung metastasis.

Collection of blood samples

Blood samples were collected from the cephalic vein from bitches in both groups in 2 different tubes (with plastic gel and ethylenediaminetetraacetic acid), 5 mL each, to perform complete blood count and evaluate blood gases and serum biochemical parameters. Blood samples were collected from the animals at the time of initial examination without any intervention. In complete blood counts including white blood cells (WBC), lymphocytes (Lym), monocytes (Mono), granulocyte (Gra), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT) parameters were performed using an automatic cell counter[1]. Moreover, the collected blood samples for blood gas parameters including pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), sodium (Na), potassium (K), lactate, blood gas parameters such as bicarbonate (HCO_3), standard bicarbonate (HCO_3std), total carbon dioxide (TCO_2), base excess in the extracellular fluid compartment (BFecf), base excess (BE), saturated oxygenion (SO_{2c}), and total hemoglobin concentration (THbc) was performed using an automatic blood gas analyzer[2]. Blood samples collected for serum biochemical analysis were centrifuged at 3000 *g* for 15 min, and the serum was separated. The separated serum samples were analyzed in an automated analyzer[3] for glucose, total protein (TP), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), urea, triglycerides, calcium (Ca), iron, iron-binding capacity, phosphorus (P), amylase, lipase, total bilirubin, albumin, magnesium (Mg), uric acid, and lactate dehydrogenase (LDH) levels.

Radiographic examination

Thoracic X-rays were performed on bitches with mammary tumors in ventrodorsal and laterolateral positions to evaluate lung metastasis. Foci larger than 6-8 mm in diameter were considered as positive for metastasis [5,36].

Extirpation of mammary tumors

Based on the laboratory findings and approval of the owners of the bitches, mammary tissues containing the tumor and lymph nodes were surgically removed. All surgical procedures were performed under general anesthesia. Catheters were inserted via

the cephalic vein to deliver propofol[4] [Propofol-PF 1%® - 6 mg/kg, IV] to induce anesthesia. The anesthetized bitches were laid face down and were orotracheally intubated. General anesthesia was maintained with isoflurane[5] [Isoflurane USP®] during the operation. The type of surgical incision and the mammary lobes to be extirpated during the operation were identified in line with the criteria specified [15]. Furthermore, ovariohysterectomy was performed on all bitches before the extirpation of mammary tumors. All bitches with mammary tumors were given cefazolin sodium[6] [Sefazol® - 25 mg/kg, IV, BID] for 7 days following the operation. Additionally, the bitches received a nonsteroidal anti-inflammatory drug butorfanol[7] [Butomidor® - 0.4 mg/kg, IV, SID] for 3 days.

Tumor size measurement and clinical stage

After the operation, the removed mammary tissues were evaluated for size and volume. Clinical staging of the tumors was performed based on the size of the primary tumor (T), nearby lymph nodes (N), and metastasis (M) in accordance with the criteria set by WHO (Table 1). The present study used this information to evaluate the size and stage of the tumors [5,35,36]. However, the study assessed the blood parameters only for bitches with a primary tumor size of 3-5 cm and > 5 cm. Bitches with a primary tumor size of < 3 cm was excluded.

Clinical staging of the tumors was, thus, based on the tumor, nodes, and metastases (TNM) score obtained according to the following system: Stage I: $T_1N_0M_0$, Stage II: $T_2N_0M_0$, Stage III: $T_3N_0M_0$, Stage IV: $T_{any}N_1M_0$, Stage V: made as $T_{any}N_{any}M_1$.

Also, tumor volume (V) using these measurements was calculated according to the following formula:

V=(W2×L) /2, where W is tumor width and L is tumor length [13,14].

Statistical analysis

The data were analyzed using SPPS 25 statistical software package[8]. 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 Shapiro-Wilk test. Pairwise comparisons were performed using the student's t-test when the prerequisites of parametric testing were met and using the Mann-Whitney U test when the prerequisites were not met. Comparison of more than 2 groups was performed with one-way analysis of variance (ANOVA) and Tukey's honestly significant difference test, and when the assumptions did not hold, the Kruskal-Wallis and Bonferroni-Dunn tests were used. The relationship between 2 continuous variables was evaluated using the Pearson's correlation coefficient, and when the prerequisites of parametric testing were not met, the Spearman correlation coefficient was used. Significance level was set at $P < 0.05$.

Table 1. Evaluation according to the criteria of the World Health Organization's primary tumor size (T), regional lymph node (N), and metastasis (M) status.

T Primary tumor	N Regional lymph node
T0 no tumor	N0 Lymph no metastasis
T1 Tumor diameter < 3 cm T1a tumor not fixed T1b tumor fixed to skin T1c tumor fixed to muscle	N1 metastasis ipsilateral lymph node N1a not fixed N1b fixed
T2 Tumor diameter 3-5 cm T2a tumor not fixed T2b tumor fixed to skin T2c tumor fixed to muscle	N2 metastasis bilateral lymph nodes N2a not fixed N2b fixed
T3 Tumor diameter > 5 cm T3a tumor not fixed T3b tumor fixed to skin T3c tumor fixed to muscle	M Metastasis status M0 no metastases M1 distant metastasis

RESULTS

Descriptive data for the bitches with mammary tumors are given in Table 2. Bitches with mammary tumors were evaluated based on the size (T) of the primary tumor in accordance with the criteria set by WHO. Accordingly, bitches with a tumor size of 3-5 cm and > 5 cm were included and with tumor size of < 3 cm were excluded from the study. Although numerous parameters specified in the Materials and Methods section were analyzed in the study, only the parameters with statistical significance were presented in the tables.

The mean age and weight (kg) of the bitches with mammary tumors were 8.42 ± 1.88 years and 19.25 ± 9.68 kg, respectively. None of the bitches with mammary tumors had been previously spayed. In bitches with mammary tumors, the primary tumor was usually located in the 3rd-5th mammary lobe and was present in both mammary chains. Some of the animals with mammary tumors were found to exhibit symptoms such as loss of appetite, dehydration, vomiting, and weight loss.

Table 3 presents the results of complete blood count, blood gas, and serum biochemical analysis for healthy bitches and those with mammary tumors (indiscriminately according to the size of the primary tumor). The findings showed that bitches with mammary tumors had decreased levels of RBC, HCT, HGB, K, TCO_2, BE, THbc, and ALT enzyme activity and elevated levels of lactate, total protein, cholesterol, triglyceride, LDL, uric acid, and ALP and LDH enzyme activities compared with the bitches in the control group ($P < 0.05$)

Table 4 presents the changes in complete blood count, blood gas, and serum biochemical parameters in bitches with mammary tumors based on primary tumor size (tumor size of 3-5 cm and > 5 cm) and in healthy ones. The results showed that bitches with a primary tumor size of > 5 cm, as compared with those in the control group, exhibited elevated WBC, lactate, TP, triglycerides, LDL, uric acid levels, and ALP and LDH enzyme activities and decreased levels of RBC, HCT, TCO_2, and ALT enzyme activity ($P < 0.05$). In addition, bitches with a primary tumor size of > 5 cm were found to exhibit the highest levels of LDH enzyme activity.

The clinical stage of the tumor was found to have a statistically significant positive correlation with triglycerides and LDL (Table 5) and a moderate negative correlation with RBC and Na levels ($P > 0.05$). Tumor volume was found to have a positive correlation with Mono, lactate, and LDH levels and a statistically significant negative correlation with MCV. Tumor volume was also found to have a moderate negative correlation with Gra, MCH, pCO_2, HCO_3, and TCO_2 levels and a moderate positive correlation with ALP enzyme activity ($P > 0.05$). Furthermore, tumor volume was found to have a strong positive correlation with LDH enzyme activity ($P < 0.05$).

This study found multiple foci of tumors in different mammary lobes in bitches with mammary tumors. Accordingly, the animals were divided into 2 groups depending on the presence of tumors in more than 1 mammary lobes (Yes or No). Table 6 presents the changes in complete blood count, blood gas, and serum biochemical parameters in bitches divided into these 2 groups. The results showed that bitches with tumors in more than 1 mammary lobe, as compared with those in the control group, had elevated levels of WBC, lactate, TP, urea, triglyceride, LDL, P, uric acid, and ALP and LDH enzyme activities and decreased levels of RBC, HCT, HGB, TCO_2, THbc, and ALT enzyme activity.

Table 2. Breed, age, weight, sterilization status, primary tumor information, and general health status of bitches with mammary tumors.

Bitches	Breed	Aged	Weight	Primer tumor location	Side of tumor(s)	Different mammary lobe	Tumor size	Clinical staging	Anorexia	Dehyd	Vomiting	WL
CMT1	Miniature Pinscher	11	5	3	R and L	Yes	> 5 cm	3	Yes	Yes	No	Yes
CMT2	Anatolian Shepherd	8	15	5	R	No	3-5 cm	2	No	No	No	No
CMT3	Cocker Spaniel	6	6	5	R	No	> 5 cm	3	No	No	No	No
CMT4	Mixed	6	28	4	L	No	> 5 cm	5	No	No	No	No
CMT5	Anatolian Shepherd	10	35	4	R and L	Yes	> 5 cm	5	Yes	Yes	No	Yes
CMT6	Siberian Husky	10	15	5	R and L	Yes	3-5 cm	4	Yes	Yes	Yes	Yes
CMT7	Cocker Spaniel	8	20	3	R	No	3-5 cm	2	No	No	No	No
CMT8	Mixed	10	12	3	R	No	3-5 cm	5	No	No	No	No
CMT9	Cocker Spaniel	11	17	2	R and L	Yes	> 5 cm	5	No	No	No	No
CMT10	English Pointer	7	19	2	R	No	3-5 cm	2	No	No	No	No
CMT11	Anatolian Shepherd	7	33	3	R	No	> 5 cm	3	Yes	Yes	No	Yes
CMT12	English Pointer	7	26	4	L	No	> 5 cm	3	No	No	No	No

CMT: canine mammary tumor; Dehyd: dehydratation; WL: weight loss.

Table 3. Changes in complete blood count, blood gases analysis, and serum biochemistry parameters (x ± SD) in healthy (control) and mammary tumor bitches (CMT).

Parameters	Control (n = 6)	CMT (n = 12)	*P*
RBC (x10⁶/μL)	7.65 ± 0.92	5.85 ± 1.46	0.015[1]*
HCT (%)	53.05 ± 6.66	41.46 ± 6.87	0.004[1]**
HGB (g/dL)	15.75 ± 2.19	12.87 ± 2.26	0.021[1]*
K (mEq/L)	4.12 ± 0.44	3.48 ± 0.63	0.024[2]*
Lactate (mmol/L)	0.98 ± 0.14	2.64 ± 1.36	0.010[1]**
TCO_2 (mmHg)	34.75 ± 2.98	21.32 ± 3.82	0.001[1]**
BE (mmol/L)	-1.35 ± 2.12	-4.77 ± 3.26	0.034[1]*
THbc (g/dL)	15.83 ± 2.19	12.06 ± 2.19	0.003[1]**
TP (g/dL)	5.09 ± 0.87	6.74 ± 1.44	0.022[1]*
Cholesterol (mg/dL)	174.45 ± 43.45	239.17 ± 41.64	0.049[2]*
ALP (U/L)	68.46 ± 47.26	132.99 ± 77.98	0.024[2]*
ALT (U/L)	39.55 ± 36.34	16.43 ± 15.35	0.005[2]**
Triglycerides (mg/dL)	56.42 ± 8.25	101.18 ± 62.73	0.024[2]*
LDL (mg/dL)	15.3 ± 9.03	54.68 ± 38.25	0.026[1]*
Uric acid (mg/dL)	0.57 ± 0.16	1.15 ± 0.85	0.010[2]**
LDH (U/L)	293.5 ± 212.68	581 ± 165.07	0.049[2]*

*$P < 0.05$; **$P < 0.01$; [1]Student's *t* Test (*t*); [2]Mann Whitney U Test (z).

Table 4. Changes in complete blood count, blood gases analysis, and serum biochemistry parameters in healthy (control) and mammary tumor dogs (CMT) according to the size of the primary tumor ($\bar{x}$ ± SD).

Parameters	Control (n = 6)	Size of primer tumors		*P*
		3-5 cm (n = 5)	> 5 cm (n = 7)	
WBC (cells/μL)	13.99 ± 5.183[A]	20.49 ± 8.339[AB]	24.4 ± 5.674[B]	0.033[2]*
RBC (x10⁶/μL)	7.65 ± 0.92[A]	6.03 ± 1.103[AB]	5.72 ± 1.756[B]	0.015[1]*
HCT (%)	53.05 ± 6.667[A]	41.14 ± 9.09[B]	41.69 ± 5.601[B]	0.004[1]**
K (mEq/L)	4.12 ± 0.449[A]	3.24 ± 0.336[B]	3.64 ± 0.77[AB]	0.038[2]*
Lactate (mmol/L)	0.98 ± 0.147[A]	2.54 ± 0.991[B]	2.71 ± 1.66[B]	0.010[1]**
TCO_2 (mmHg)	34.75 ± 2.98[A]	21.1 ± 4.363[B]	21.47 ± 3.743[B]	0.001[1]**
BE (mmol/L)	-1.35 ± 2.125[A]	-5.44 ± 4.144[B]	-4.29 ± 2.721[AB]	0.034[1]*
THbc (g/dL)	15.83 ± 2.191[A]	12.16 ± 2.141[B]	11.99 ± 2.394[B]	0.003[1]**
TP (g/dL)	5.09 ± 0.879[A]	6.56 ± 1.317[AB]	6.88 ± 1.626[B]	0.022[1]*
ALP (U/L)	68.46 ± 47.26[A]	135.09 ± 30.141[AB]	231.49 ± 62.832[B]	0.049[2]*
ALT (U/L)	39.55 ± 36.343[A]	14.64 ± 6.878[B]	17.71 ± 19.901[B]	0.024[2]*
Triglycerides (mg/dL)	56.42 ± 8.259[A]	98 ± 20.288[AB]	103.46 ± 32.5[B]	0.047[2]*
LDL (mg/dL)	15.3 ± 9.039[A]	45.5 ± 41.715[AB]	61.23 ± 37.457[B]	0.026[1]*
Uric acid (mg/dL)	0.57 ± 0.162[A]	0.84 ± 0.433[AB]	1.38 ± 1.039[B]	0.008[2]**
LDH (U/L)	293.5 ± 212.685[A]	239.4 ± 150.237[A]	825 ± 195.215[B]	0.049[2]*

*$P < 0.05$; **$P < 0.01$; [1]ANOVA (F); [2]Kruskal Wallis Test (H); [A,B]Different letters or combinations of letters on the same row indicate statistically significant difference ($P < 0.05$).

Table 5. Correlation between complete blood count, blood gases analysis, and serum biochemistry parameters according to clinical staging and volume of tumor in bitches with mammary tumors.

Parameters	Clinical Stage (r)	Parameters	Tumor volume (r)
WBC	0.343[1]	Lym	0.319[1]
Mono	0.349[1]	Mono	0.592[1]*
RBC	-0.572[1]	Gra	-0.506[1]
Platelets	0.306[1]	MCV	-0.619[1]*
pCO2	0.388[1]	MCH	-0.528[1]
Na	-0.456[1]	RDW	0.435[1]
K	0.407[1]	pCO_2	-0.432[1]
HCO_3	0.464[1]	Lactate	0.695[1]*
HCO3std	0.449[1]	HCO_3	-0.419[1]
BEecf	0.487[1]	TCO_2	-0.465[1]
BE	0.457[1]	BEecf	-0.342[1]
ALT	-0.318[1]	BE	-0.324[1]
Triglycerides	0.592[2]*	TP	-0.392[1]
LDL	0.588[2]*	ALP	0.545[1]
Phosphorus	0.352[1]	LDL	0.361[2]
Amylase	0.337[1]	Lipase	-0.305[1]
		LDH	0.920[1]**

*$P < 0.05$; **$P < 0.01$; [1]Pearson's Correlation Coefficient; [2]Spearman Correlation Coefficient.

Table 6. Complete blood count, blood gases analysis, and serum biochemistry parameters (x ± SD) of bitches with mammary tumors in multiple lobes.

Parameters		Tumors in multiple mammary lobes		
	Control (n = 6)	No (n = 8)	Yes (n = 4)	*P*
WBC (cells/μL)	13.99 ± 5.183[A]	17.9 ± 7.377[B]	32.52 ± 14.52[B]	0.014[1]*
RBC (x10^6/μL)	7.65 ± 0.92[A]	6.57 ± 0.767[AB]	4.41 ± 1.539[B]	0.001[1]**
HCT (%)	53.05 ± 6.667[A]	42.65 ± 7.041[B]	39.08 ± 6.794[B]	0.012[1]*
HGB (g/dL)	15.75 ± 2.195[A]	13.31 ± 2.201[B]	11.98 ± 2.442[B]	0.048[1]*
K (mEq/L)	4.12 ± 0.449[A]	3.43 ± 0.292[B]	3.58 ± 1.13[B]	0.038[2]*
Lactate (mmol/L)	0.98 ± 0.147[A]	2.7 ± 1.438[B]	2.53 ± 1.413[B]	0.007[2]**
TCO_2 (mmHg)	34.75 ± 2.98[A]	21.06 ± 4.224[B]	21.83 ± 3.375[B]	0.001[1]**
THbc (g/dL)	15.83 ± 2.19[A]	12.48 ± 1.523[B]	11.23 ± 3.287[B]	0.010[1]**
TP (g/dL)	5.09 ± 0.879[A]	6.93 ± 1.102[B]	6.97 ± 0.099[B]	0.049[1]*
Urea (mg/dL)	25.49 ± 4.984[A]	28.24 ± 10.424[AB]	47.19 ± 14.124[B]	0.050[1]*
ALP (U/L)	68.46 ± 27.26[A]	142 ± 41.134[B]	144.97 ± 59.208[B]	0.047[2]*
ALT (U/L)	39.55 ± 36.343[A]	20.26 ± 17.317[B]	8.78 ± 6.881[B]	0.024[2]*
Triglycerides (mg/dL)	56.42 ± 8.259[A]	88.49 ± 31.335[B]	126.58 ± 36.039[B]	0.044[2]*
LDL (mg/dL)	15.3 ± 9.039[A]	40.9 ± 31.826[B]	82.23 ± 38.541[B]	0.017[2]*
Phosphorus (mg/dL)	5.56 ± 0.924[A]	6 ± 1.08[AB]	7.88 ± 2.366[B]	0.048[1]*
Uric acid (mg/dL)	0.57 ± 0.162[A]	1.15 ± 1.059[B]	1.17 ± 0.281[B]	0.008[2]**
LDH (U/L)	293.5 ± 112.685[A]	535.5 ± 198.158[AB]	603.75 ± 290.806[B]	0.049[2]*

*$P < 0.05$; **$P < 0.01$; [1]ANOVA (F); [2]Kruskal Wallis Test (H); [A,B]Different letters or combinations of letters on the same row indicate statistically significant difference ($P < 0.05$).

DISCUSSION

Mammary tumors are the most common type of neoplasm in bitches and, thus, cause serious problems in veterinary medicine [4]. In many studies, mammary tumors are considered an independent prognostic factor for mammary tumors in bitches [15,16,31]. Tumors are significantly correlated with better prognosis compared with larger tumors [5]. Based on this finding, this study investigated the effect of size, volume, and stage of mammary tumors in bitches on some blood parameters and the correlation between them.

This study found that bitches with mammary tumors, compared with healthy ones, had decreased levels of RBC, HGB, and HCT in complete blood count and decreased THbc in blood gas analysis. Moreover, bitches with a tumor size of > 5 cm and tumors in multiple mammary lobes had a more pronounced decrease in RBC and THbc, and there was also a negative correlation between the clinical stage of the tumor and RBC [18], and have also reported that the RBC levels were lower in bitches with mammary tumors compared with the healthy ones [12]. Moreover, have associated mild-to-moderate nonregenerative (absence of reticulocytosis), normocytic, normochromic anemia with mammary tumors in bitches [11]. This observation is due to cytokines (interleukin-1 and tumor necrosis factor alpha) released by tumor cells. These cytokines sequester iron, reduce the half-life of erythrocytes (IL-1), and also reduce the release of erythropoietin (TNF and IL-6). In addition, previous studies have found microangiopathic hemolytic anemia and immune-mediated hemolytic anemia in mammary carcinoma [29]. Moreover, nonregenerative anemia in patients with cancer is thought to be caused by anemia of chronic disease, also known as anemia of inflammation [6].

Although this study found no statistical difference in WBC values between healthy bitches and those with mammary tumors (13.99 ± 5.18 and 22.77 ± 11.99, respectively), it found significantly increased WBC values, especially when the tumors increased in size and when the tumor tissues were present in multiple mammary lobes ($P < 0.05$). Moreover, although there was no difference in WBC values between healthy bitches and those with mammary tumors, the values were outside the reference values in bitches with mammary tumors. There was also a moderate positive correlation between the clinical stage of the tumor and the WBC value ($P > 0.05$) [19] and have reported that the WBC levels were higher in canine mammary carcinomas than in the control group and that neutrophils were the dominant WBC type [12]. Neutrophilia is thought to be caused by regions of intense inflammation and ulceration in the tumor tissue. Moreover, previous reports have associated the increased presence of neutrophils with poor prognosis and have observed that the neutrophils increased more markedly in patients with large tumor tissues, metastasis, and death [12,19].

Blood gas analysis showed a significant increase in lactate in bitches with mammary tumors, with the values reaching the highest level in bitches with a tumor size of > 5 cm. There was also a positive correlation ($r = 0.695$) between tumor volume and lactate levels. Similarly, serum biochemical analysis found higher LDH enzyme activity in bitches with mammary tumors compared with those in the control group. Also, LDH enzyme activity was the highest in bitches with a tumor size of > 5 cm and with tumors in multiple mammary tissues. Furthermore, there was a strong positive correlation between tumor volume and LDH enzyme activity ($r = 0.920$). Previous data [7] have reported increased levels of lactate, whereas other studies [3,30] have reported increased LDH enzyme activity in bitches with mammary tumors. In addition, one study has reported a significant correlation between LDH enzyme activity and the staging of the tumor and the progression of the disease [3]. Rapid proliferation and high metabolic needs in cancer cells lead to an increase in LDH. LDH is an enzyme responsible for catalyzing the reversible conversion of pyruvate to lactate [23]. Glycolysis is abnormally high in tumor cells [17]. High glycolysis leads to increased lactate production, which stimulates the proliferation of malignant oxygenated cells. This process supports angiogenesis and suppresses natural and acquired immunity and is associated with poorer prognosis and malignity [7,8,10].

Serum biochemical analysis performed in this study also found changes in some parameters in bitches with mammary tumors. First, the levels of total protein were found to be increased in bitches with mammary tumors compared with those in the control group, which reached the highest level in bitches with a tumor size of > 5 cm and with tumors in multiple mammary lobes. Malignant mammary tumors can cause conditions such as proteinemia in humans and bitches [25,39]. Indeed, previous data [1] has reported increased levels of total protein in women with breast

tumors, and other studies [24,25] have reported the same observation in bitches with mammary tumors. The increase in total protein levels in mammary tumors is due to an increase in the level of albumin and other proteins, such as globulins. Albumin concentrations may vary under tumor-associated oxidative stress. Hypergammaglobulinemia may also develop in cases of acute or chronic inflammation [1,6,25].

When liver enzymes were analyzed, ALP enzyme activity was found to have increased in bitches with mammary tumors compared with those in the control group. Furthermore, ALP enzyme activity was found to have reached the highest level in bitches with a tumor size of > 5 cm and was positively correlated with tumor volume. Several other studies have also reported increased levels of ALP enzyme activity in bitches with mammary tumors [13,20,21]. The increase in serum ALP in some mammary tumors is thought to be due to the excessive osteoblastic activity of neoplastic cells, which is associated with osseous transformation [20]. Indeed, intratumoral osseous transformation is expected in bitches with a tumor size of > 3cm and in advanced stages of the disease [21]. It has also been observed that tissue-ALP activity increased in myoepithelial cells of both normal and neoplastic mammary glands. Therefore, increasing levels of ALP in the serum may also indicate localized production of isoenzyme by tumor cells, similar to the human's Regan isoenzymes [13,15,20,21].

ALT (formerly serum glutamic pyruvic transaminase) is a liver-specific enzyme in dogs. Its highest cellular concentration is found in the cytosol. Therefore, elevated levels of ALT are associated with serious, acute, and diffuse hepatocellular necrosis [28]. Aerobic glycolysis occurs at high rates in the cancer cells owing to high proliferation [9]. These pathological events cause AST to be activated more than ALT in fast-growing cancer tissues. Therefore, ALT levels are lower in highly invaded cells than in less invaded ones. At the same time, oxidative stress and inflammation may occur in relation to cancer development, which may also cause liver dysfunction [41]. The present study found that ALT enzyme activity decreased in bitches with mammary tumors compared with those in the control group. The ALT values reached their lowest levels in bitches with tumors in multiple mammary lobes and were negatively correlated with the clinical stage of the tumor. Similar to the present study, previous data [2] have reported decreased levels of ALT in cases of malignant mammary tumors compared with the control group. However, studies on canine mammary tumors have reported that an association cannot be established between ALT enzyme activity and tumor [11,24]. The decrease in ALT enzyme activity is thought to be due to decreased parenchyma, altered intrahepatic structure, and sustained limited damage [37]. However, further research is needed on this topic.

Moreover, the present study found increased levels of cholesterol, triglycerides, and LDL in bitches with mammary tumors compared with healthy ones. Triglyceride and LDL levels were found to be the highest in bitches with a primary tumor size of > 5 cm and with tumors in multiple mammary lobes; also, there was a positive correlation between triglycerides and LDL and the clinical stage of the tumor. Previous study [38] has also reported increased levels of cholesterol and triglyceride in bitches with mammary tumors. Also, it has observed that triglycerides and LDL reached higher levels in more malignant (aggressive) tumors [7]. In addition, one study with mice has reported that hypercholesterolemia stimulated the tumor to be more aggressive and acted as a greater predisposing factor for metastasis [26]. Furthermore, studies in women have reported a significant increase in triglycerides, cholesterol, and LDL in breast cancers [27,33].

CONCLUSIONS

This study found that bitches with mammary tumors exhibited significant changes in their blood parameters (complete blood count, blood gas analysis, and serum biochemistry). Furthermore, significant correlations were observed between tumor size and clinical stage, and these parameters were more markedly affected in bitches with larger tumors and tumors in multiple mammary lobes. The results obtained from this study may contribute to the development of approaches to the diagnosis, prediction of prognosis, and treatment of canine mammary tumors.

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