RESEARCH ARTICLE

Investigation of some biochemical parameters in bitches with malignant mammary tumors

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Malign meme tümörlü köpeklerde bazı biyokimyasal parametrelerin araştırılması

Öz

Amaç: Bu çalışmada, meme tümörü olan dişi köpeklerde biyokimyasal parametrelerin cut-off değeri belirlenerek teşhis için biyobelirteç olarak kullanılabilirliği araştırıldı.

Gereç ve Yöntem: Kliniğimize rutin ovariohisterektomi için getirilen (kontrol grubu, n = 14) ve meme tümörü olan (CMT grubu, n = 14) dişi köpekler çalışmaya dahil edildi. Biyokimyasal parametrelerden albümin (ALB), alkalin fosfataz (ALP), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), gama-glutamiltransferaz (GGT), total protein (TP), direkt bilirubin (DB), total bilirubin (TB), kreatin, kreatin kinaz-N-asetil-sistein (CK-NAK), üre, fosfor (P), magnezyum (Mg) ve kalsiyum (Ca) düzeyleri otomatik bir biyokimya analizör ile ölçüldü.

Bulgular: ALP (p: 0,048), ALT (p < 0,000), AST (p: 0,043), GGT (p: 0,013), TP (p: 0,010), DB (p: 0,043), TB (p: 0,018), kreatin (p < 0,000), P (p < 0,000) ve CK-NAC (p < 0,000) düzeyleri CMT grubunda kontrol grubuna göre daha yüksek anlamlı bulundu. Mg (p < 0,000) düzeyi kontrol grubunda daha yüksek olmasına rağmen, gruplar arasında ALB, üre ve Ca düzeyleri için istatistiksel olarak fark bulunmadı (p > 0,05). ALP, ALT, AST, GGT, TP ve kreatin düzeylerinin, sırasıyla 0.656, 0.831, 0.610, 0.792, 0.942 ve 0.974 eğri altında kalan alan değerleri ile meme tümörlerinin teşhisi için kullanılabileceği görüldü.

Öneri: ALP, ALT, AST, GGT, TP ve kreatinin köpek meme tümörü için yardımcı tanı araçları olabileceği kanısına varıldı.

Anahtar kelimeler: Biyokimyasal parametreler, köpek, meme tümörleri

Abstract

Aim: This study, the cut-off point of biochemical parameters in bitches with mammary tumors was determined and its usability as a biomarker for diagnosis was investigated.

Materials and Methods: Bitches that presented to our clinic for routine ovariohysterectomy (control group, n = 14) and suffer from mammary tumors (CMT group, n = 14) were included in the study. Biochemical parameters including albümin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total protein (TP), direct bilirubin (DB), total bilirubin (TP), creatine, creatine kinase-N-acetyl-cysteine (CK-NAC), urea, phosphorus (P), magnesium (Mg), and calcium (Ca) concentrations were measured with an automated chemistry analyzer.

Results: The ALP (p: 0.048), ALT (p < 0.000), AST (p: 0.043), GGT (p: 0.013), TP (p: 0.010), DB (p: 0.043), TP (p: 0.018), creatine (p < 0.000), P (p < 0.000), and CK-NAC (p < 0.000) concentrations were found to be higher in the CMT group than in the control group. Although Mg (p < 0.000) concentration was higher in the control group, there was no statistical significance for ALB, urea, and Ca concentrations among the groups (p > 0.05). It was seen that ALP, ALT, AST, GGT, TP, and creatine concentrations could be used to diagnose mammary tumors, with area under the curve (AUC) values of 0.656, 0.831, 0.610, 0.792, 0.942, and 0.974, respectively.

Conclusion: It was concluded that ALP, ALT, AST, GGT, TP, and creatine could be helpful diagnostic tools for canine mammary tumors.

Keywords: Biochemical parameters, bitch, mammary tumors

Introduction

Cancer rates have risen dramatically in both humans and animals in recent years. Human breast cancer (HBC) is women's second most common cancer. Unfortunately, most HBCs are cancerous. The type of tumor, significant nuclear and cellular pleomorphism, the mitotic index, necrotic areas, peritumoral and lymphatic invasion, and regional lymph node metastases are all used to determine the malignancy (Akram et al 2017, Kuppusamy et al 2019). The risk of disease recurrence and metastases to other organs, such as the liver or lungs, are biologically and physiopathologically comparable between canine mammary tumors (CMTs) and HBCs (Abdelmegeed and Mohammed 2018). CMTs occur at a rate of 25-50% and are 50% malignant. Cancer has the potential to spread to nearby lymph nodes and internal organs (Sorenmo 2003, Salas et al 2015, Zheng et al 2022).

The cause of CMTs is still unknown, but significant risk factors such as hormonal, nutritional, and genetic factors have been identified. Sex hormones are among the most critical risk factors for tumor formation (Sorenmo et al 2000, Chang et al 2009, Beauvais et al 2012). The CMT is a multifactorial disease, meaning that more than one thing causes and worsens it. For example, tumor behavior is affected by epidemiological, clinical, and histological factors. Even though mammary tumors can happen in any bitch breed, they seem to happen more often in pure breeds (Baştan and Zonturlu 2002, Altan et al 2013, Burrai et al 2020). Mammary tumors in bitches are more common in middle-aged and older bitches, with a high death rate between 8 and 11 years of age (Erer and Kıran 1993, Sorenmo et al 2011). Also, a study found that performing ovariohysterectomy on bitches before their first estrus may reduce the chance of tumor formation. A more recent retrospective study found that performing ovariohysterectomy with resection of benign tumors can reduce the number of new tumors (Schneider et al 1969, Kristiansen et al 2013). Canine mammary tumors are the most commonly diagnosed tumors in non-spayed bitches (Benavente et al 2016). Adenocarcinoma, papillary carcinoma, solid carcinoma, complex carcinoma, and carcinosarcoma are the most common types of CMTs. Fibroadenomas, ductal papillomas, benign mixed tumors, and simple adenomas are the most common non-malignant mammary tumors. Two or more tumor types are frequently found in different mammary glands of the same bitch (Sontas et al 2009, Reddy et al 2009, Tavasoly et al 2013).

The stage, size, histological type, and grade of the tumor, as well as nearby and distant metastasis, all have a significant impact on the clinical prognosis of CMT. In CMT, it is recommended to perform a prognostic evaluation in addition to tumor size and stage, as well as serum biochemistry. Because CMT can cause significant changes in both the general condition of the bitch and the serum biochemistry, evaluating these parameters provides information that can help veterinarians make decisions regarding treatment and plan treatment (Sorenmo 2003; Cassali et al 2012; Nunes et al 2019). Therefore, the present study aimed to define a cut-off point by assessing the applicability of biochemical indices in bitches with mammary tumors and to evaluate the applicability of biochemical indices as diagnostic biomarkers in bitches.

Material and Methods

Animals

Bitches with mammary tumors and regular ovariohysterectomies were brought to the Kastamonu University Veterinary Faculty Obstetrics and Gynecology Clinic to be used in the study. Bitches that presented to our clinic for routine ovariohysterectomy (control group, n = 14) and suffer from mammary tumors (CMT group, n = 14) were included in the study. Bitches with mammary tumors of different breeds (German Shepherd (n = 4), Golden Retriever (n = 2), Yorkshire Terrier (n = 1), Maltese Terrier (n = 3), and Mixed breed (n = 4)), were included in the study.

Blood sample collection

Non-anticoagulant-containing vacutainers were used to collect blood samples. Vena cephalica antebrachial samples were collected into tubes without anticoagulant for biochemical testing. After being separated by centrifugation at 5,000 rpm for 8 minutes, the serum was placed in a freezer at -80°C until it was analyzed.

Surgical management

Subcutaneous Atropine sulfate (Vetaş Atropin 0.2%, Vetaş, Türkiye) at a dose of 0.02 mg/kg was administered 10 min before the anesthesia as premedication. After that, the general anesthesia protocol was carried out using the Xylazine hydrochloride (2 mg/kg, intramuscularly) (Rompun® 2%, Bayer, Türkiye) and Ketamine hydrochloride (10 mg/kg, intramuscularly) (Ketasol 10%, Richterpharma, Austria) combination as practiced in the previous study (Safak et al 2021). During the surgery, all of the dogs were able to breathe on their own. During the anesthesia, the patient was given 5 ml/kg/h of Lactate Ringer's solution (Ringer Laktat, Polifarma, Türkiye) through an intravenous. During the surgery, heart and respiratory rates and body temperatures were recorded. After a mastectomy, tissue samples were procured for the histopathological examination.

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Histopathology examination

The pathological diagnosis of CMTs was performed as follows: tissues were embedded in paraffin in 10% neutral buffered formalin, and sections (5 μm) were split but also routinely stained with hematoxylin-eosin (H&E) for histopathological analysis. Histopathological classification was applied according to Goldschmidt et al (2011). The Pena system was used to determine the histological grade based only on the malignancy epithelial component. Classified as grade I, grade II, and grade III according to the pena system (Pena et al 2013).

Serum biochemistry

Biochemical parameters including albümin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total protein (TP), direct bilirubin (DB), total bilirubin (TB), creatine, creatine kinase-N-acetyl-cysteine (CK-NAC), urea, phosphorus (P), magnesium (Mg), and calcium (Ca) concentrations were measured with an automated chemistry analyzer (Gesan Chem 200, Italy).

Statistical analysis

The Shapiro-Wilk test was used to examine the variables in terms of the parametric test assumptions. The Mann-Whitney U test was also used to explore the differences between groups in biochemical parameter indices for variables that did not have a normal distribution. On the other hand, the Student's t-test was applied to make pairwise comparisons of the bitch's age, weight, and tumor sizes. Comparisons between the two groups were considered statistically significant if the p value was less than 0.05. Statistical analyses were performed using IBM SPSS Version 22.0 (Statistical Package for the Social Sciences for Windows SPSS 22.0 Edition for Windows, Chicago, Illinois, USA).

Receiver operating characteristic (ROC) curve analysis was used to determine the best threshold value for diagnosing biochemical parameters in dogs with mammary tumors and their sensitivity and specificity. For each biochemical index, analysis of ROC curves was performed to detect CMT. The sensitivity, specificity, and area under the curve (AUC) were worked out for each variable. The AUC values are interpreted as stated in the literature (Tümer et al 2022); >0.90 meant high accuracy, 0.70–0.90 meant moderate accuracy, 0.5–0.7 meant low precision, and ≤0.5 meant the test was not valid.

Table 1. Bitch's age, weight, and tumor sizes (Mean ± SD)			
Item	Control (n = 14)	CMT (n = 14)	p value
Age (years)	5.95 ± 1.21	9.19 ± 0.41	< 0.001
Weight (kg)	35.16 ± 2.73	22.26 ± 1.49	< 0.05
Size of tumor (cm)	NM	6.57 ± 0.36	

NM, no mass; SD, Standard deviation; CMT, canine mammary tumors

Table 2. Tumors are classified as grades I, II, and III and histologic types				
Histologic diagnosis	Histological grade			%
	I	II	II	
Simple carcinoma (n: 6)	2	1	3	42.85
Complex carcinoma (n: 3)	2	1	0	21.43
Carcinosarcoma (n: 1)	0	0	1	7.14
Osteosarcoma (n: 2)	0	1	1	14.29
Malignant mixed tumor (n: 2)	2	0	0	14.29
Total (n: 14)	6	3	5	100

Table 3. Comparison of biochemical parameters between groups (median=min - max)			
Parameters	Control (n = 14)	CMT (n = 14)	p value
	Median (Min - Max)	Median (Min - Max)	
ALB(g/dL)	3.46 (2.05 - 4.16)	3.99 (2.74 - 4.49)	-
ALP (U/L)	64.00 (39.00 - 105.00)	70.00 (44.00 - 243.00)	0.048
ALT (U/L)	24.00 (13.00 - 41.00)	32.00 (26.00 - 47.00)	<0.000
AST (U/L)	34.80 (23.40 - 53.60)	35.00 (30.00 - 91.00)	0.043
GGT (U/L)	8.00 (7.00 - 12.00)	11.00 (10.00 - 17.00)	0.013
TP(g/dL)	6.40 (5.70 - 7.50)	7.70 (7.00 - 8.60)	0.010
DB (mg/dL)	0.08 (0.06 - 0.13)	0.09 (0.08 - 0.19)	0.043
TB(mg/dL)	0.15 (0.09 - 0.25)	0.18 (0.13 - 0.29)	0.018
Creatine (mg/dL)	1.00 (0.24 - 1.47)	1.81 (1.20 - 1.95)	<0.000
CK-NAC (U/L)	195.00 (133.00 - 640.00)	350.00 (139.00 - 601.00)	<0.000
Urea (mg/dL)	34.00 (28.00 - 41.00)	39.00 (29.00 - 63.00)	-
P (mg/dL)	3.10 (2.28 - 4.81)	4.66 (4.00 - 5.31)	<0.000
Mg (mg/dL)	3.10 (2.70 - 3.90)	2.34 (2.00 - 3.00)	<0.000
Ca (mg/dL)	12.20 (11.80 - 14.00)	12.00 (11.00 - 15.00)	-

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; TP, total protein; DB, direct bilirubin; TB, total bilirubin; CK-NAC, creatine kinase-N-acetyl-cysteine; P, phosphorus; Mg, magnesium; Ca, calcium; CMT, canine mammary tumors; -, >0.05.

Table 4. The receiver operating characteristic curve analysis for determination of the critical thresholds for biochemical parameters as a predictor of mammary tumors status between bitches with mammary tumors and the control group						
Parameters	Optimized Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
ALP (U/L)	> 127	42.86	100	100	57.89	0.656
ALT (U/L)	> 26	100	63.64	77.78	100	0.831
AST (U/L)	> 52	42.86	90.91	85.71	55.56	0.610
GGT (U/L)	> 10	100	45.45	70.00	100	0.792
TP(g/dL)	> 7	100	72.73	82.35	100	0.942
Creatine (mg/dL)	> 1.2	100	90.91	93.33	100	0.974

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; TP, total protein; AUC, Area Under the Curve; PPV, Positive Predictive Value; NPV, Negative Predictive value
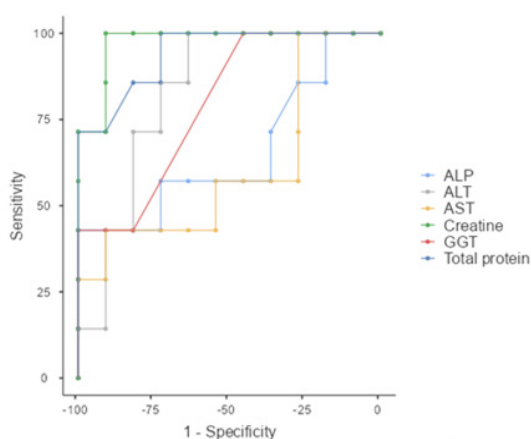

Figure 1. The Receiver operating characteristics curve analysis determined the thresholds for ALP, ALT, AST, GGT, creatine, and total protein concentrations for predicting the canine mammary tumor

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Results

Bitches with mammary tumors, mean 9.19 ± 0.41 years old and weight 22.26 ± 1.49 kg. The control group, with an average age of 5.94 ± 1.21 years and a weight of 35.16 ± 2.73 kg, brought for ovariohysterectomy are shown in Table 1.

The 14 malignant tumors were graded as follows: 6 (42.86%) grade I, 3 (21.43%) grade II, and 5 (35.71%) grade III (Table 2).

The ALP (p: 0.048), ALT (p < 0.000), AST (p: 0.043), GGT (p: 0.013), TP (p: 0.010), DB (p: 0.043), TB (p: 0.018), creatine (p < 0.000), CK-NAC (p < 0.000), and P (p < 0.000) concentrations were significantly higher in the CMT group than in the control group. Although Mg (p < 0.000) concentration was higher in the control group, there was no statistical significance for ALB, urea, and Ca concentrations among the groups (p > 0.05) (Table 3).

The results of the ROC curves analysis for the determination of critical Cut-off, specificity, sensitivity, Positive Predictive Value, Negative Predictive Value, and AUC values are presented in Table 4. ALP, ALT, AST, GGT, TP, and creatine concentrations are the best predictors for the diagnosis of CMT, with AUC values of 0.656, 0.831, 0.610, 0.792, 0.942, and 0.974, respectively. The ROC curve for the crucial Cut-off between the CMT and the control groups is shown in Figure 1.

Discussion

Breast cancer is the most common cancer in humans around the World (Harbeck and Gnan 2017), and CMT is common cancer in bitches (Reddy et al 2009). Mammary tumors in bitches are often used as the first step in research on breast cancer. CMT is one of the most common tumors, and it is a significant threat to the life and health of bitches. It is also hard to treat and hard to keep from happening. Even so, veterinarians are doing much research to improve the early diagnosis and life expectancy of bitches with CMTs. Much of the study was done on the factors that lead to CMT formation (Gunnes 2017, Seung et al 2021, Ariyarathna et al 2021).

Mammary tumors are more prevalent in bitches older than five, with the typical age being 9 to 11 years (Baştan and Zonturlu 2002, Zheng et al 2022). In the study, the mean age of bitches with mammary tumors was determined as 9.19 ± 0.41 following the researchers' report (Günay-Uçmak and Güvenç 2019). CMT can spread by entering the blood and lymph circulation. It is described as malignant cells shed by the tumor or its metastases circulating in the patient's peripheral circulation. The existence of these cells is a crucial prognostic factor for tumor recurrence and disease-related mortality, as well as a marker of metastatic

potential (Ignatiadis et al 2008). Cancer frequently spreads to the liver, lungs, lymph nodes, and bones. Even if cancer only seems to be in one organ, liver metastasis is usually a sign of widespread cancer with a terrible prognosis (Atalay et al 2003). The ability of a screening test to find a disease can be made more sensitive or specific if it is used with more than one test. So, tumor markers combined with serum liver enzymes may make tests for finding liver metastases more sensitive and accurate (Hellmen et al 1993, Kaszak et al 2022). GGT, AST, ALP, ALT, lactate dehydrogenase (LDH), and CA15-3 concentrations were considerably higher in patients with liver metastases than those without (Cao and Wang 2012). Results were observed in the patient's liver function tests when they presented, with GGT and ALP being the enzymes that were most frequently found to be high. In addition, patients' AST levels were more than as high as the maximum value considered normal (O'Reilly et al 1990). Both serum carbohydrate antigen (CA) 15.3 and LDH concentrations correlated with disease stage. These findings support the use of CA15.3 as a prognostic factor for CMT (Campos et al 2012).

In this study, ALP, ALT, AST, GGT, TP, DB, TB, creatine, and CK-NAC concentrations were more significant in the CMT group compared to the control group. It was found that the concentration of ALP was higher in bitches with CMT than in those in the control group. The same study discovered that ALT concentration decreased in CMT group compared to control group (Satilmis et al 2022). Bitches with malignant mammary tumors had significantly higher serum ALP activity compared with control bitches (Karayannopoulou et al 2006). In another study (Raghavendra-Srikanth et al 2021), biochemical parameters such as ALT, AST, and creatine did not differ significantly between CMT-affected and control bitches. In the present study, however, both ALT and ALP concentrations were higher in the CMT group than in the control group.

In humans, the combined tumor and biochemical parameters' sensitivity and specificity could be employed as indicators in screening for breast-liver metastases. Therefore, a diagnostic test was conducted to see if LDH and GGT concentrations might be used to detect liver metastases. Because diagnostic indices of LDH and GGT were maximum at 174 U/L and 32 U/L, respectively, for detecting liver metastases, these Cut-off thresholds were used. LDH and GGT sensitivity values were 81.0% and 68.2%, respectively, while specificity levels were 69.5% and 82.5%, respectively (Cao and Wang 2012). In this study, it is considered that activities of >127 (U/L) ALP, >26 (U/L) ALT, >52 (U/L) AST, >10 GGT, >7 (g/dL) TP, and >1.2 (mg/dL) creatine for CMT can be used in the preliminary diagnosis of these diseases. However, the overall test performance of these findings is moderate, and their practical use for diagnosing these diseases is uncertain. Considering the association of high biochemical values and

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bitches with CMT, some biochemical parameters remain essential for testing research hypotheses, especially when a more objective parameter is needed for CMT.

Conclusion

In conclusion, this study showed that ALP, ALT, AST, GGT, TP, and creatine concentrations could be helpful diagnostic tools for CMT. It is predicted that easily accessible, simple, and low-cost indices can be used as practical markers for diagnosing mammary tumors in bitches. Moreover, the findings of this study may aid in the development of perspectives to the diagnosis, prognosis prediction, and treatment of CMT.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Ethical Approval

Approval for the study was obtained from the Kastamonu University Animal Experiments Local Ethics Committee (E-16498365-000-2200131368).