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Research Article

B-mode Echotexture Analysis and Color Doppler Sonography in Canine Mammary Tumors

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Abstract

Forty-one mammary gland tumors from twenty eight bitches were used for the study. Ultrasonographic examinations of tumor masses were performed before surgical excision and a quadratic region-of-interest (ROI) was chosen randomly on B-mode tumor images for the echotexture analyses. All tumors were evaluated histopathologically after surgery. Contrast (CONT), Mean Gradient (MG), Mean Value (MV), Homogeneity (HOM), Entropy (ENTR) and Gray Value (GV) parameters were used for the texture analyses of ultrasonographic images. Ultrasonographic image characteristics were additionally evaluated by the following macroscopic patterns: tumor shape, invasion of tumor to surrounding tissue, tumor border sharpness, echogenicity of tumor, hyperechogenic artifact, anechogenic artifact, and shadow around tumor. After B-mode ultrasonographic examination, Pulsatility Index (PI), Resistive Index (RI), Peak Systolic Flow Velocity (Vmax) and Number of Color Pixel (CP) parameters were evaluated by means of color Doppler sonography. Statistical analysis of the HOM and GV parameters indicated that there was a significant difference between benign (3.10 and 1.14) and malignant tumors (1.54 and 0.57; P<0.01). Besides, a significant difference was found between images of Malignant-Mixed Tumors (MMT) and Benign-Mixed Tumors (BMT) with regard to CONT and HOM (p< 0.001). In addition, MV was significantly higher in malignant tumors in comparison to the benign cases (P<0.05). A significant negative correlation was found between tumor size and MV in malignant tumor and adenocarcinoma cases (-0.991/ P<0.05; -0.999/P<0.01, respectively). On the other hand, there was a positive correlation between tumor size and GV (0.961/P<0.05) in malignant tumors.

Keywords: *Canine, Mammary tumor, Doppler, Echotexture analysis, Ultrasound*

Köpek Meme Tümörlerinde B-Mode Ekodesen Analizi ve Renkli Doppler Ultrasonografi

Özet

Çalışma için 28 dişi köpeğe ait olan 41 meme tümörü dokusu kullanılmıştır. Cerrahi eksizyon öncesi tümörlü kitleler B-Mode ultrasonografik muayene ile incelenmiş, görüntüler digital olarak kayıt edilmiş ve ekodesen analizi için bu görüntüler üzerinde rastgele olarak dörtlü inceleme alanları (Region of Interest) seçilmiştir. Tüm tümörlü dokular, cerrahi eksizyon sonrası histopatolojik olarak incelenmiştir. Ultrasonografik resimlerin yapısal analizleri için, Kontrast (CONT), Ortalama Gradyan (MG), Ortalama Değer (MV), Homojenite (HOM), Entropi (ENTR) ve Gri Değer (GV) parametreleri kullanılmıştır. Ultrasonografik resimler ek olarak tümör kitlesi, tümör şekli, tümörün çevre dokulara invazyonu, tümör sınır keskinliği, tümörün ekojenitesi, hiperekojenik artefakt, anekojenik artefakt ve tümör etrafındaki gölgelenme gibi makroskopik parametreler açısından da değerlendirilmiştir. B-Mod ultrasonografik muayenenin ardından, renkli Doppler ile Pulzatil İndeks (PI), Rezistif İndeks (RI), Pik Sistolik Akım Hızı (Vmax) ve Renkli Piksel Sayısı (CP) parametreleri değerlendirilmiştir. İstatistiki analizler sonucunda HOM ve GV parametreleri açısından, benign (3.10 ve 1.14) ve malign (1.54 ve 0.57; P<0.01) tümörler arasında önemli farklar bulunmuştur. Ayrıca CONT ve HOM parametreleri açısından Malign Miks Tümörler (MMT) ve Benign Miks Tümörler (BMT) arasında önemli farklar (P<0.001) bulunmuştur. Ek olarak, MV malign tümörlerde, benign tümörlere göre önemli düzeyde (P<0.05) yüksek bulunmuştur. Tümör büyüklüğü ve MV arasında, malign tümörlerde ve adenokarsinomlarda önemli düzeyde negatif korrelasyon saptanmıştır (sırası ile -0.991/P<0.05; -0.999/P<0.01). Diğer yandan malign tümörlerde tümör büyüklüğü ve GV arasında pozitif korrelasyon saptanmıştır (0.961/P< 0.05).

Anahtar sözcükler: *Dişi köpek, Meme tümörü, Doppler, Ekodesen analizi, Ultrason*

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INTRODUCTION

Mammary tumors are one of the most common tumor types reported in female dogs [1,2]. Incidence of malignant mammary tumors among all mammary tumors is within a range of 41 to 68 per cent [3-6]. Histopathological examination is required for the diagnosis of tumor type [7], however B-mode and color Doppler ultrasonography were studied to discriminate between benign and malignant tumors [8]. Studies conducted with two dimensional B-mode ultrasonography showed that classifying malignant and benign mammary tumors was not possible in veterinary medicine [9,10]. In contrast, Marquardt et al.[11] compared B-mode ultrasonographic images including shape, size, anechogenic areas and echogenicity of surrounding tissue with histologic findings and determined that some ultrasonographic parameters may have an important role in classifying malignant tumors but diagnosing tumors with low malignancy might be impossible [12]. To obtain further assurance, more detailed examinations are necessary. Improvements of the differentiability of the different changes of canine mammary tumors can be achieved by refined and improved ultrasonographical examination technologies [13]. Color Doppler ultrasonography is used in order to assess tumor vascularity in human medicine for the purpose of antivascular therapy [14,15]. Studies performed in human medicine revealed a significant correlation among echogenicity, echostructure and mamma sonographic findings [16]. Echostructure analysis is carried out by examination of regions of interest (ROI) by digital B-mode ultrasonography and consecutive calculation of special parameters with a computerized programme [17].

Computed echostructure analysis was performed on acquired B-mode ultrasonographic images for diagnostics purposes in humans previously [18,19]. Garra et al.[20] and Bader et al.[16] investigated the differences between mammary tumors and other tissue types by using texture analysis and echogenicity parameters.

The objective of the present study was to compare B-mode image echostructure and color Doppler ultrasonographical analyses with histopathologic findings to figure out, whether it is possible to discriminate between benign and malignant canine mammary tumors.

MATERIAL and METHODS

Animal Grouping and Image Acquisition

Twenty eight mongrel bitches with a total of 41 mammary tumors or tumor-like lesions brought to the Clinic of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Ankara (TR) were used in the study. The age of the bitches was in the range of 5 to 14 years.

The clinical status of all bitches was determined according to standard procedures. The general condition of the bitches with mammary tumors was determined to be moderately or severely affected. Detailed examination of mammary glands was performed by inspection and palpation. The absence or presence of lung metastases was evaluated by thoracic radiography. The mammary masses were examined by B-mode ultrasound (Esaote AU5; 7.5 MHz, linear transducer). Mastectomy was performed under general anesthesia. The animals were premedicated with 0.045 mg/kg of atropine sulphate (Belladone®, Alke, İstanbul, Turkey) and sedated with 2 mg/kg of xylazine HCL (Alfazyne® %2, Egevet, İzmir, Turkey). Following sedation, 10 mg/kg ketamine HCL (Alfamine® %10 Egevet, İzmir, Turkey) was applicated i.v. The tumor and mammary tissue were sent to the pathology laboratory for routine processing (Department of Pathology, Faculty of Veterinary Medicine, University of Ankara, Tr). Tissue specimens were immediately fixed in formalin (10%) and were embedded in paraffin using standard techniques as described by Luna, (1968) [21]. Tissue sections (5-6 µm) were cut and stained with haematoxylin-eosin (HE). Canine mammary tumors were classified as benign and malignant tumors according to Moulton [22]. Malignant tumors were divided into two groups: Adenocarcinomas and malignant mixed tumors (MMT).

B-mode ultrasonographical images were inspected macroscopically according to the method described by Marquardt et al.[12] and Gonzalez de Bulnes et al.[23]. Tumor shape (regular or irregular), invasion of tumor to surrounding tissue (clear or not clear), tumor border sharpness (sharp or not sharp), echogenicity of tumor (hypoechogenic, hyperechogenic, anechogenic, or mixed), echo display of tumor (homogeneous or heterogeneous), hyperechogenic artifact (present or absent), anechogenic artifact (present or absent), and shadow around tumor (present or absent) were examined.

Tumor dimensions were divided into three groups by using a formula for an area of an ellipse (AE; 3.14 X a/2 X b/2; a= Longer axis; b= Shorter axis) [24] on ultrasonographical images: Group 1 = ≤ 200 mm^2, group 2 = > 200 mm^2 - ≤ 500 mm^2, group 3 = > 500 mm^2

Analysis of ultrasound images was performed using a series of custom-developed computer algorithms optimized for ultrasonography (Synergyne©, Version 2.8, WHIRL, Saskatoon, Sask., Canada) on a Sun Sparc Station 20 computer (Sun Microsystems, Mt. View, CA, USA). Digitized images of mammary tumors were divided into four equal quadrants. On each quadrant, a quadratic region-of-interest (ROI) was chosen randomly on B-mode tumor images and echotexture analyses were done using a customized program (PEPE v1.0, German Cancer Research Center, Heidelberg, Germany) as described by Schmauder et al.[25]. For the computer-assisted analysis of B-mode imaging, echotexture of the tissues using the parameters mean gray level (MGL), mean gradient (MG), homogeneity (HOM), entropy (ENTR), contrast (CONT) and gray value (GV) was evaluated *(Fig. 1)*. These parameters were defined

by Allison et al.[26] and Moss et al.[27] as: *Mean Gray Level* (Arithmetical average grey level of all pixels in picture, defines the brightness), *Mean Gradient* (Variations in grey values of neighbor pixels, defines microtexture of sample), *Homogeneity* (Uniformity of grey value combination of neighbor pixels in defined matrix, defines either micro- or macrotexture of sample), *Entropy* (A measure of the uniformity of matrix values), *Contrast* (A measure of how many large grey-level differences are present in the ROI), *Gray Value* (The brightness of pixels in a digitized image).

The largest sections of tumors were visualized and measured on B-mode ultrasonography for the best evaluation. The assessment of material was done according to number of tumors as some dogs suffered from more than one mass. Computer-assisted analyses were therefore performed on 286 ROI (regions of interest) from malignant, 48 ROI from benign and 118 ROI from adenocarcinomas of 86 tumor images from 28 bitches. A total of 14 bitches were examined by power Doppler due to vascularization of masses and parameters of RI, PI and Vmax (n=21) were investigated. Moreover, 58 Color Doppler images were obtained from 28 dogs by monitoring the highest colorful blood velocities.

The blood flow of tumor vessels was quantified by the Doppler pulsatility index (PI), resistive index (RI) and peak systolic flow velocity (Vmax) (Esaote AU5; 7.5-10 MHz, linear transducer) *(Fig. 2)*. While PI is defined as (peak systolic velocity-end diastolic velocity)/time averaged velocity, RI is defined as (peak systolic velocity - end diastolic

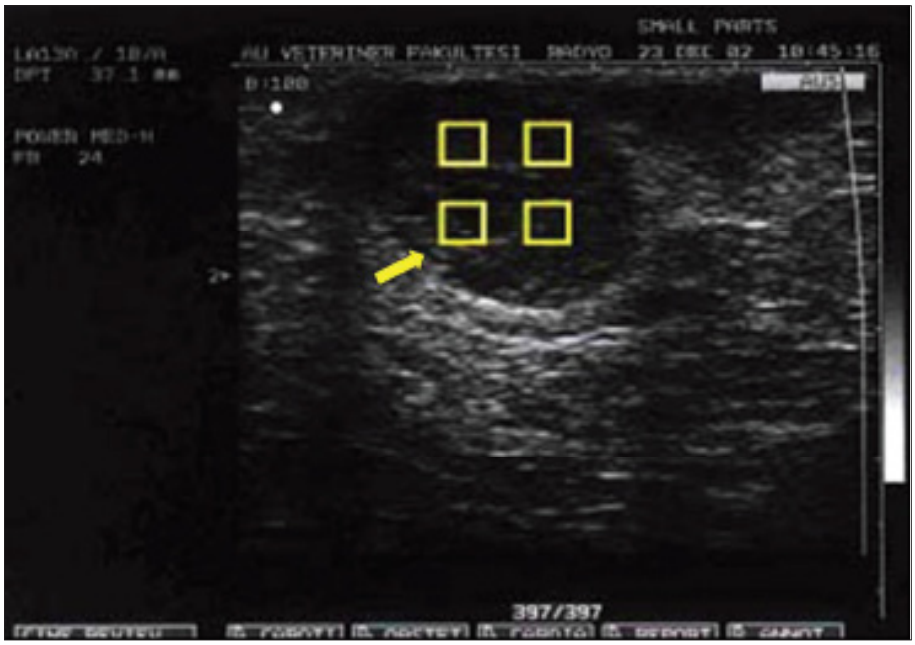

Fig 1. Computer-assisted analysis of B-mode imaging

Şekil 1. B-Mod görüntülemede bilgisayar destekli analiz

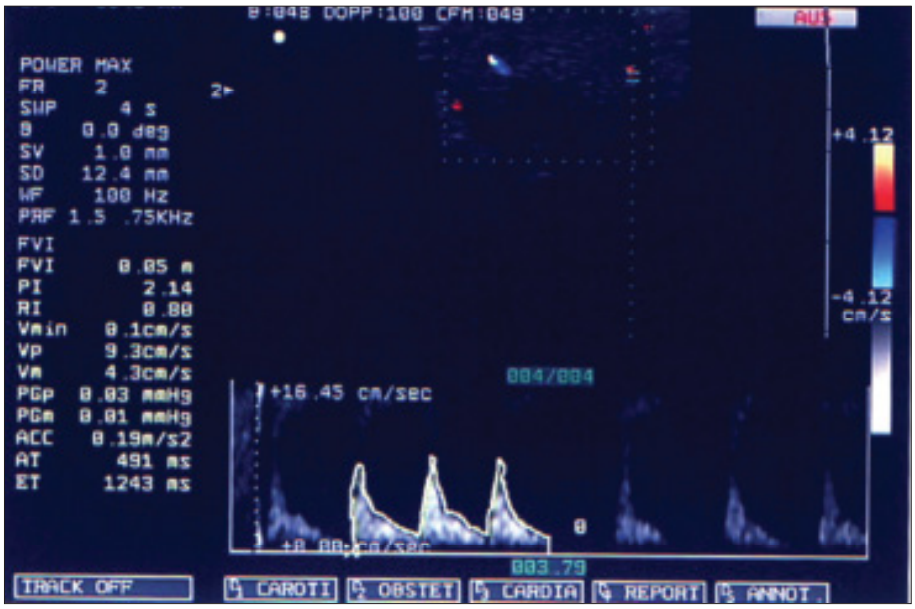

Fig 2. The blood flow analysis of a tumor vessel by color Doppler

Şekil 2. Tümör kan damarında renkli Doppler ile kan akış analizi

velocity)/peak systolic velocity [8]. The vascularization of the mammary tumors was visualized in Color-Angio-Mode and quantified using a computer program (Adobe Photoshop software 5.0, Adobe Systems) to determine the number of color pixels [28].

Statistical analysis was performed by SPSS® (Version 17.0, SPSS Inc, Chicago, USA). All data are given as means ± standard deviation descriptive statistic; the normality and homogeneity of variances were assessed for all variables tested by means of a "Shapiro-Wilks" test and "Bartlett-Box"test. For normally distributed data, differences between groups were compared using "one-way ANOVA". For not normally distributed data, the "Mann–Whitney-U" test was used for comparison between two groups. "Kolmogorov-Smirnov Z-test" was used for the difference between more than two groups.

RESULTS

The results of histopathological examination and numbers of tumors are given in *Table 1*.

Based on the histopathological diagnosis, the tumors were divided into 2 groups: Benign tumors (n=5) and malignant tumors (n=36). A total of 286 regions-of-interest (ROI) from malignant tumors and 48 ROI from benign tumors were obtained. Echotexture parameters are represented on *Table 2*.

Significant differences in echostructure parameters between the different groups of tumors were found *(Table 2)*. HOM, GV and MV were significantly higher in malignant than in benign tumors (P<0.01 and P<0.05). Similarly in MMT, the average CONT and HOM were significantly higher than in BMT (P<0.001 each), and among the malignant tumors, adenocarcinomas had significantly higher GV than the MMT (P<0.001). But there was no significant difference between tumor types concerning the Doppler parameters (P>0.05) *(Table 3)*.

In *Table 4*, the relation between malignant tumor sizes and echostructure parameters is given.

In malignant cases, a negative correlation was found between tumor size and MV (r=-0.995; P<0.05), and a positive correlation between tumor size and GV (r=0.961, P<0.05) *(Table 4)*. No significant difference was calculated when the average values were compared between groups. In *Table 5*, the relation between tumor size of AC and echostructure parameters is given.

In adenocarcinoma cases, a negative correlation was determined between tumor size and MV (r= -0.999; P<0.01), whereas in benign tumors, there was no significant correlation between tumor size and any echostructure parameters.

Table 1. *Postoperative histopathological diagnosis and numbers of mammary tumors*
Tablo 1. *Postoperatif histopatolojik teşhis ve meme tümörü sayısı*

Tumor Type	Benign Tumors	Malignant Tumors	
		Adenocarcinomas	Malignant Mixed Tumors
Benign mixed tumor	4		
Fibro-mixo-lipo adenoma	1		
Tubular adenocarcinoma		2	
Tubulopapillary adenocarcinoma		1	
Complex adenocarcinoma		1	
Papillary cystic adenocarcinoma		3	
Solid adenocarcinoma		2	
Malignant mixed tumor			27
N	5	9	27

Table 2. *Echostructure analysis of regions of interest (ROI) on B-Mode images of malignant, benign, malignant and benign mixed tumors.*
Tablo 2. *Malign, benign, malign ve benign karma tümörlere ait B-Mod resimler üzerindeki inceleme alanlarının (ROI) ekodesen analizi*

Tumor Pathology	CONT (X ± SD)	MG (X ± SD)	MV (X ± SD)	HOM (10^{-3}) (X ± SD)	ENTR (X ± SD)	GV (X ± SD)
Malignant (N=286)	98.43±65.10	31.76±12.60	77.39±46.27	3.10±2.08	2.65±0.49	1.14±1.06
Benign (N=48)	162.72±154.62	27.58±18.08	65.28±44.78	1.54±1.25	2.41±0.69	0.57±0.46
P	>0.05	>0.05	<0.05	<0.01	>0.05	<0.01
MMT (N=168)	101.11±67.46	31.73±12.33	75.74±43.04	3.15±2.98	2.64±0.43	0.70±0.58
BMT (N=48)	162.72±154.62	27.58±18.08	65.28±44.78	1.54±1.25	2.41±0.69	0.57±0.46
P	<0.001	>0.05	>0.05	<0.001	>0.05	>0.05
MMT (N=168)	101.11±67.46	31.73±12.33	75.74±43.04	3.15±2.98	2.64±0.43	0.70±0.58
AC (N=118)	94.61±61.68	31.80±13.04	79.74±50.60	2.42±2.32	2.66±0.57	1.49±1.41
P	>0.05	>0.05	>0.05	>0.05	>0.05	<0.001

****MMT:*** *Malignant mixed tumor,* ***BMT:*** *Benign mixed tumor,* ***AC:*** *Adenocarcinoma,* ***CONT:*** *Contrast,* ***MG:*** *Mean Gradient,* ***MV:*** *Mean Value,* ***HOM:*** *Homogeneity (X 10^{-3}),* ***ENTR:*** *Entropy,* ***GV:*** *Gray Value (X 10^{-3}); P<0.05 indicates statistically significant difference*

Table 3. *Relationship between tumor types and Doppler parameters (PI, RI, Vmax, CP)*
Tablo 3. *Tümör tipi ve Doppler parametreleri (PI, RI, Vmax, CP) arasındaki ilişki*

Tumor Type	PI (X ± SD) (n)	RI (X ± SD) (n)	Vmax (X ± SD) (n)	CP (X ± SD) (n)
Malignant	1.85±0.30 (16)	0.68±0.12 (16)	7.15±4.34 (16)	64910.53±51431.88 (51)
Benign	1.83±0.34 (5)	0.63±0.10 (5)	6.64±2.76 (5)	62386.69±52217.46 (13)
P	>0.05	>0.05	>0.05	>0.05
MMT	1.83±0.34 (11)	0.69±0.13 (11)	6.53±3.50 (11)	65718.72±39947.3 (32)
BMT	1.83±0.34 (5)	0.63±0.10 (5)	6.64±2.76 (5)	62386.69±52217.46 (13)
P	>0.05	>0.05	>0.05	>0.05
MMT	1.83±0.3 (11)	0.69±0.13 (11)	6.53±3.50 (11)	65718.72±39947.3 (32)
AC	1.93±0.23 (5)	0.67±0.13 (5)	8.52±6.06 (5)	207293.30±234725 (19)
P	>0.05	>0.05	>0.05	>0.05

PI: *Pulsatility index,* ***RI:*** *Resistive index,* ***Vmax:*** *Maximum systolic flow (cm/s),* ***CP:*** *Number of color pixel;* ***MMT:*** *Malignant mixed tumor,* ***BMT:*** *Benign mixed tumor,* ***AC:*** *Adenocarcinoma; P<0.05 indicates statistically significant difference*

Table 4. *Relationship between malignant tumor sizes and echostructure parameters*
Tablo 4. *Malign tümör büyüklükleri ve ekodesen parametreleri arasındaki ilişki*

Groups	MV (X ± SD)	MG (X ± SD)	CONT (X ± SD)	HOM (x10^{-3}) (X ± SD)	ENTR (X ± SD)	GV (X ± SD)
Group 1 (n=56); ≤200 mm^2 106.53±52.59	88.28±40.76	32.27±9.81	117.73±70.15	4.95±4.54	2.68±0.30	0.50±0.25
Group 2 (n=48); > 200 mm^2 ≤ 500 mm^{2}303.30±58.64	78.12±41.84	30.70±13.21	90.76±60.70	3.64±3.48	2.54±0.48	0.52±0.33
Group 3 (n=64); > 500 mm^2 760.84±398.25	62.36±43.27	31.71±13.80	89.80±68.03	7.30±7.01	2.51±0.45	1.52±1.51
R	-0.995; P< 0.05	-0.133; P< 0.05	-0.750; P> 0.05	0.791; P> 0.05	-0.734; P>0.05	0.961; P< 0.05

n: *number of B-mode images of each tumor;* ***AE:*** *area of an ellipse (mm^2);* ***CONT:*** *Contrast,* ***MG:*** *Mean Gradient,* ***MV:*** *Mean Value,* ***HOM:*** *Homogeneity (X 10^{-3}),* ***ENTR:*** *Entropy,* ***GV:*** *Gray Value (X 10^{-3}); P<0.05 indicates statistically significant difference*

Table 5. *Relationship between adenocarcinoma tumor sizes and echostructure parameters*
Tablo 5. *Adenokarsinom tümör büyüklükleri ve ekodesen parametreleri arasındaki ilişki*

Groups	MV (X ± SD)	MG (X ± SD)	CONT (X ± SD)	HOM (x10^{-3}) (X ± SD)	ENTR (X ± SD)	GV (X ± SD)
Group 1 (n=18); ≤200 mm^2 128±29.82	147.40±35.55	38.58±4.64	109.27±22.95	1.93±1.24	2.84±0.13	0.75±0.22
Group 2 (n=8); > 200 mm^2 ≤ 500 mm^2 386.3±0.0	137.41±20.18	43.81±2.58	197.60±33.84	1.01±0.43	3.08±0.05	2.61±0.64
Group 3 (n=92); > 500 mm^2 1830.27±677.24	61.49±38.60	29.43±13.66	82.78±59.90	1.87±1.68	2.50±0.60	1.53±1.51
R	-0.999; P<0.01	-0.873; P>0.05.	-0.568; P>0.05	0.319; P>0.05	-0.844; P>0.05	0.048; P>0.05

AE: *area of an ellipse (mm^2);* ***CONT:*** *Contrast,* ***MG:*** *Mean Gradient,* ***MV:*** *Mean Value,* ***HOM:*** *Homogeneity (X 10^{-3}),* ***ENTR:*** *Entropy,* ***GV:*** *Gray Value (X 10^{-3}); P<0.05 indicates statistically significant difference*

Results of the comparison between benign and malignant tumors according to macroscopical B-mode image evaluation are given in *Table 6*.

Macroscopical evaluation of B-mode ultrasonographic images revealed no significant differences between benign and malignant tumors (P>0.05).

DISCUSSION

Mammary tumors are one of the most common neoplasms in bitches. Reports indicate that the incidence of mammary neoplasms comprises a range of 22.9 to 52 per cent of all canine tumors [2,29]. Histopathological examination is essential to exhibit criteria of tumor dignity. Nevertheless there is no consensus on classification of canine mammary tumors due to variety of mammary tumors, though there are many proposals for histological and histogenetical classification [7,30,31]. Researchers mostly use the classification of canine and feline mammary tumors prepared by the World Health Organization (WHO) and adapted from human tumor classification systems [32,33]. However, the fact that myoepithelial cells in canine mammary tumors contribute to neoplastic proliferation

Table 6. *Comparison of benign and malignant tumors according to macroscopical B-mode image evaluation*
Tablo 6. *Makroskopik B-Mode resim değerlendirmesine göre, benign ve malign tümörlerin karşılaştırılması*

USG Appearance	Parameters B-mode Pictures	Benign (n=12) n; (%)	Malignant (n=74) n; (%)	P
Tumor shape	Regular	12/6 (50)	74/37(50)	>0.05
	Irregular	12/6 (50)	74/37 (50)	>0.05
Invasion of tumor to surrounding tissue	Clear	12/4 (33.3)	74/32 (43.2)	>0.05
	Not clear	12/8 (66.7)	74/42 (56.8)	>0.05
Tumor border sharpness	Sharp	12/9 (75)	74/50 (67.6)	>0.05
	Not sharp	12/3 (25)	74/24 (32.4)	>0.05
Echogenicity of tumor	Mixed	12/8 (66.7)	74/48 (64.9)	>0.05
	Hypoechogenic	12/2 (16.7)	74/13 (17.6)	>0.05
	Anechogenic	12/0 (0)	74/5 (6.8)	>0.05
	Hyperechogenic	12/2 (16.7)	74/8 (10.8)	>0.05
Structure of tumor	Homogeneous	12/4 (33.3)	74/26 (35.1)	>0.05
	Heterogeneous	12/8 (66.7)	74/48 (64.9)	>0.05
Hyperechogenic artifact	Existent	12/1 8.3)	13 (17.6)	>0.05
	Absent	12/11 (91.7)	61 (82.4)	>0.05
Anechogenic artifact	Existent	12/3 (25)	10 (13.5)	>0.05
	Absent	12/9 (75)	64 (86.5)	>0.05
Shadow existence around tumor	Existent	12/0 (0)	4 (5.4)	>0.05
	Absent	12/12 (100)	70 (94.6)	>0.05

USG *= ultrasonographical*

differs canine tumors from tumors of other animals and humans [34]. In this study, pathological findings including tubular adenocarcinoma, tubulopapillary adenocarcinoma, solid adenocarcinoma and malignant mixed tumor were evaluated as malignant tumors, and fibro-myxo-lipo adenomas as benign tumors. Since the aim of the study was to create a supportive method which might help to make a decision for surgery, malignant and benign tumors were compared, as well as malignant and benign mixed tumors. Besides tumors diagnosed as tubular adenocarcinoma, tubulopapillary adenocarcinoma and complex mammary adenocarcinoma were classified under the heading of "adenocarcinoma".

Histopathological examination to diagnose mammary gland tumors is obligatory [2]. Nevertheless, other diagnosis techniques should be taken into consideration [6,35].

In human studies, differences between mammary tumor and other types of tissues (necrosis in adipose tissue, proliferative mastopathies and cysts) detected by means of B-mode echotexture analysis were reported [16,20]. Tumors were classified as benign or malignant tumors dependant on the appearance of tissues. Because of this differentiation, a significant decrease in the number of breast biopsies was achieved [20].

In veterinary medicine, computer-assisted texture analysis programs were developed to evaluate changes in the ovarium and endometrium during the estrous cycle [25,36]. Morphological and echotexture attributes were correlated with CL function, and the luteal tissue heterogeneity correlated to circulating progesterone concentrations [37].

Results of the present study indicate that based on homogeneity and gray value, computer-assisted texture analysis may be a helpful diagnostic method to differentiate benign from malignant tumors among the here investigated tumor types. Homogeneity defines the level of uniformity in ROIs on B-Mode ultrasound images. Presence of lower gray value combination accompanied with equal distribution means an increase in homogeneity, the contrary means a decrease [38]. Due to the presence of bone, cartilage and fatty tissues in benign mixed tumors [7], relative high gray value combination occurs on B-Mode ultrasound images that appears to be more heterogeneous. However, lower gray value combination and more homogeneous distribution on ultrasound images were observed when malignant tumor types were evaluated without further categorization, since a malignant mixed tumor is formed by mesenchymal or epithelial components; this might coincide with cyst formation in cases of papillary cystic adenocarcinoma, in case of solid carcinoma little stroma is present [30,33]. Some studies in human medicine demonstrated that fibrocystic lesions, fat necrosis and cystic structures can be differentiated from malignant tumors by using echostructure analysis [16].

Previous studies demonstrated a correlation between canine mammary tumor size, tumor type and prognosis [8,39]. Nyman et al.[8] reported that malignant tumors were larger than benign ones. In the present study, no correlation between echostructure parameters and the size of benign tumors was found, however, between MMT and adenocarcinomas, echostructure parameters based on tumor size differentiated. Therefore, the fact that echostructure parameters do not change in larger masses might be a characteristic for diagnosis of benign tumors.

In this study, in malignant tumors, the mean value parameter decreased with increasing size of tumors, whereas the gray value increased. Mean value parameters in adenocarcinomas showed similar changes. In benign tumors, no correlation was found between tumor size and echotexture parameters, therefore particularly mean value and gray value might be important parameters to differentiate benign from malignant tumors.

Rapid increase in canine mammary tumor size may be a malignancy criterion [22,40]. This feature is similar to human mammary tumors [41,42]. Decrease in mean value parameters is observed together with a decrease in image brightness. Mean gray value parameters varied by tumor size and resolution of images [43,44] that leads to a non-homogenous appearance. Rapidly grown malignant tumors (adenocarcinoma, sarcoma) are reported to have morphologically irregular surfaces with a bluish color and nodular composition on palpation contrary to histologically benign tumors (adenoma, fibroma, mixed tumors) [45]. Schoenrock [46] reported that the incidence of spongy nodules or spongy smooth structures was 10 per cent. These clinical findings explain the heterogeneous echostructure appearance of malignant tumors.

The results of the present study obtained from both Doppler parameters and morphological analysis of ultrasonographical images demonstrated that no differences were found between malignant and benign tumors ($P>0.05$). It has been reported in some studies that ultrasonographical noninvasive methods were unable to provide useful information for differentiation between benign and malignant tumors [9,10,13]. On the other hand Marquardt et al.[11] indicated that shape, relation with surrounding tissue, echogenic rim, internal echogenicity, internal echographic pattern, posterior acoustical enhancement, sound attenuation, shadowing were important criteria for the evaluation of canine mammary gland tumors. Marquardt et al.[12] reported that the percentage of preoperative accurate diagnosis was 77.4 in malignant tumors, and 91.9 in benign tumors. Nyman et al.[8] demonstrated that echogenicity, tumor border shape, acoustical shadowing, number of vessels to the tumor and the total vascular flow were important diagnostic criteria for discrimination between malignant and benign canine mammary tumors. Bastan et al.[47] reported that tumor size, shape, border irregularity, echotexture, internal echogenicity and acoustic transmission parameters were useful for evaluation of canine mammary gland tumors. Different results of many studies performed by visual analysis of B-mode ultrasonographical images may be due to ultrasonographical technique, categorization, the number of material, individual differences in evaluation. However, the facts that medullar carcinomas might be comprehended as benign tumors [48,49], and sonographical dorsal or ventral shadowing were missed by certain authors [50,51] show that B-mode ultrasonography does not provide enough information on tumor characteristics.

In humans, radical mastectomies remained the standard until the 1970s, when a new understanding of metastasis led to perceiving cancer as a systemic illness as well as a localized one, and more sparing procedures were developed that proved equally effective. Mammary tumours, clinically not yet apparent, were sometimes not detected by ultrasonographical examination. The demonstration of primary multiplicity of canine mammary tumours via ultrasound is only possible for already clinically diagnosed tumours. A final diagnosis of the primary multiplicity in an early stage is only possible when using histological examination. A differentiation between benign and malign tumours was not possible with ultrasonographical examination. An exact diagnosis could only be made by histological examination [13]. Our findings revealed that tumor shape, invasion of tumor to surrounding tissue, anechogenic artifact, hyperechogenic artifact and other parameters were ultrasonographically detectable in both malign and benign tumors, therefore these parameters proved not to be useful for differentiation between benign and malign mammary tumors.

Studies with color Doppler sonography showed remarkable differences in peak systolic flow velocity between malignant and benign tumors [52,53]. In a study conducted in humans [54], preoperatively carcinoma diagnosis with color pixel intensity was achieved in 60% of cases and accurate diagnosis was possible in 91.9%. However, carcinoma diagnosis was correct in 92% and diagnosis was accurate in 78% of cases when analyzed by color Doppler sonography. There was no difference concerning microvasculature structures between non-metastatic malignant and benign canine tumors [55]. Both B-Mode ultrasonography and color Doppler sonography techniques were inadequate to diagnose tumor characteristics [56]. In practice, there are some structural differences between human and canine mammary tumors. In human medicine, epithelial-myoepithelial carcinoma tumor types and benign-malignant canine myoepithelial proliferations do not appear [57]. The ineffectiveness in tumor differentiation by using ultrasonography and color Doppler presumably might be due to this anatomical and histological diverseness of canine mammary tumors.

In conclusion, these results demonstrate that it is possible to differentiate between benign and malignant

tumors by means of echostructure analysis, whereas B-mode or Doppler ultrasonography techniques are insufficient to distinguish malignancy from benignity in canine mammary tumors. Furthermore, echostructure analysis corresponding to B-mode image acquisition might be an oncoming perspective.

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