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Strain elastography is a useful tool in the evaluation of canine mammary neoplasms

Muzaffer Onur Şeran[1*], Zeynep Günay Uçmak[2] and Çağatay Tek[2]

[1]Vetkadıköy Veterinary Clinic, Istanbul, Türkiye
[2]Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye

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ABSTRACT

The aim of this study was to evaluate the diagnostic performance of strain elastography in the assessment of mammary neoplasms. Twenty-three dogs and a total number of 57 mammary neoplasms were included in this study. The elasticity score and strain ratio were measured using elastography recordings for comparison of histopathology results in mammary neoplasms. The mean elasticity scores were 1.66±0.15 for the benign neoplasms and 3.33±0.16 for the malignant tumors (P<0.001). The optimal cutoff value was found to be between 2 and 3 (2.5), with values of 71.4% sensitivity and 93.3% specificity. The mean strain ratios were calculated 1.85±0.21 for the benign neoplasms and 3.76±0.23 for the malignant tumors (P<0.001). In the analysis of strain ratio measurements, the optimal cutoff value was found to be 2.15 with 90.5% sensitivity and 80.0% specificity. Analyses with both methods of the malignant epithelial tumors in different grade groups were not statistically different (P>0.05). There was no significant difference found in distinguishing malignant mixed tumors and malignant epithelial tumors (P>0.05). The results of this study showed that, strain elastography is a highly valuable tool in differential diagnosis of mammary neoplasms in bitches.

Key words: tsukuba elasticity score; elastography; strain ratio; mammary tumor; dog

Introduction

Elasticity is defined as the change in the shape of a material that can be returned to its original dimensions after the removal of the force that has been externally applied. Qualitative and quantitative elastography applications have been widely used for the evaluation of diseased and healthy tissues since the development of ultrasonographic elastography (UE), which has enabled the measurement of tissue elasticity and hardness by means of ultrasound. The results of a previous study (NOWICKI and DOBRUCH-SOBCZAK, 2016) with neoplastic tissues showed that malignant lesions are harder than benign lesions, and hard tissues have lower degrees of strain.

Strain elastography (SE) was the first UE technique to be developed (OPHIR et al., 1991). In

*Corresponding author:
Muzaffer Onur Şeran, DVM, MSc, PhD, Vetkadıköy Veterinary Clinic, 34710, Istanbul, Türkiye, email: onur_seran@hotmail.com

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this technique, qualitative results can be obtained with the elasticity scoring method (ITOH et al., 2006; BAMBER et al., 2013; GENNISSON et al., 2013; İLERISOY YAKUT et al., 2015; SHIINA et al., 2015; NOWICKI and DOBRUCH-SOBCZAK, 2016; DIETRICH et al., 2017; SIGRIST et al., 2017). The best-known elasticity scoring method is the Tsukuba elasticity score classification, which was developed through studies on breast neoplasms. According to this method, the relationship of the hardness of the lesions with the surrounding tissues is evaluated visually and qualitatively with color patterns (ITOH et al., 2006). Semi-quantitative values can be calculated with the strain ratio method in SE. The strain ratio calculation tool of the elastography software compares the elasticity of the lesion with the elasticity of surrounding normal tissues for reference (HOSKINS, 2011; DIETRICH et al., 2017). A ratio greater than 1 indicates that the lesion is harder and has a lower degree of strain than the reference tissue. High strain ratios suggest malignancy (DIETRICH et al., 2017; SIGRIST et al., 2017).

As a result of the widespread use of elastography in human healthcare, many UE studies have been reported in the veterinary literature on different tissues (GLIŃSKA-SUCHOCKA et al., 2013; WHITE et al., 2014; FELICIANO et al., 2015; JEON et al., 2015; LONGO et al., 2018). To the best of our knowledge, there are no reports evaluating the SE elasticity scoring and strain ratio in female dogs with mammary neoplasms in terms of benignancy and malignancy. The aim of this study is to investigate the diagnostic value of SE by comparing the Tsukuba elasticity score classification and strain ratio measurement with the histopathology results as the gold standard in mammary neoplasms.

Materials and methods

All animal procedures were carried out in accordance with the approval of the Local Ethical Committee at Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine (Approval number: 04/08/2017-295824).

Twenty-three female dogs (3-16 years old; 6-45 kg) from different breeds that were presented to Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology between 2017-2019 and a total number of 57 mammary neoplasms, were included in this study.

All animals were clinically examined. Mammary glands were initially evaluated by inspection and palpation. Three-view thoracic radiography (Orex PcCR 1417 and Viztek diagnostic Imaging Programme, USA), biochemical tests (Catalyst One Chemistry Analyzer, Idexx, USA) and complete blood count tests (Procyte Dx Hematology Analyzer, Idexx, USA) were performed in order to evaluate the preoperative anesthesia risks for each animal.

All ultrasound examinations were performed by the same operator by portable ultrasonography (USG) (Sonoscape S9, Sonoscape Medical Corporation, China) equipped with a 4-16 Mhz linear probe. The dogs were positioned in dorsal recumbency. Initially, B-mode USG was performed for each mammary gland. Afterwards, each scanned mammary gland was evaluated with the SE module of the USG device (Sonoscape S9, Sonoscape Medical Corporation, China).

When taking images with the elastography module, the strain map was selected, in which shades of blue encode hard tissues, shades of red denote the soft tissues, and the shades of green indicate tissues of intermediate hardness (Fig. 1). The region of interest (ROI) boundaries were determined in the image to include the skin tissue at the top and the thoracic or abdominal muscle wall at the bottom (ITOH et al., 2006; BARR et al., 2015). Then, minimal pre-compression was created perpendicular to the tissue through the transducer, to reveal the superimposed image of the elastography map on the ultrasound screen (Fig. 1). After the ideal pre-compression was generated, repeated compression and relaxation movements were performed 1-2 times a second without interrupting the transducer-skin contact (OPHIR et al., 1991). Whether sufficient compression was applied or not was followed in the strain curve of the SE module (Fig. 1). For adequate compression, the curves created should be in the range of 0.5-1 in the strain curve. After taking at least 5 consecutive curves with negative and positive maxima in the range of 0.5-1, elastography recordings were made in video format for measurements. This recording procedure was repeated 3 times for each neoplasm to calculate the mean strain ratio and the mean elasticity score.

Measurements were taken from the images corresponding to the midpoints of the maximum and minimum compressions on the strain curve from the elastography recordings (BAMBER et al., 2013). Initially, the Tsukuba elasticity score was measured from the elastography recordings. According to this system, Score 1 means that the lesion has similar elasticity to the surrounding tissue, and it is soft coded. Score 2 means that most of the lesion is soft coded, but shows hard foci with blue-green tessellation in some places. Score 3 means that the hard circumference of the center of the lesion is coded soft and the hard part does not reach to the lesion border. Score 4 indicates that the lesion is hard and some hard areas have reached to the lesion border. In score 5, the lesion is hard and the hard areas exceed the lesion boundaries (ITOH et al., 2006) (Fig. 2).

The second measurement made using the elastography recordings in the study was the strain ratio. The muscle was chosen as the reference tissue because, from the literature it is known that there is a difference between the adipose density in the thoracic, abdominal and inguinal mammary glands of female dogs (SORENMO et al., 2011). Thus, it was ensured that the results obtained from the strain ratio measurements were not affected by the gland density difference. The strain values of the lesion and the reference tissue were initially measured with the SE module as percentages. Then, the strain value of the reference tissue was divided by the strain value of the lesion by using the SE module, and the strain ratio was calculated (Fig. 3).

All mammary glands obtained after bilateral mastectomy were histopathologically analyzed at Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, in the Department of Pathology.

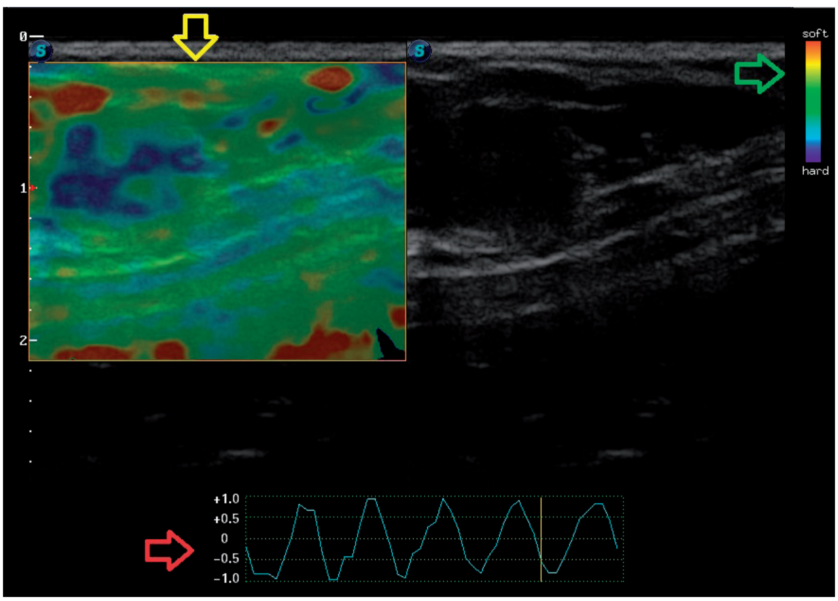

Fig. 1. Components of a strain elastography image

Yellow arrow: superimposed image of the elastography map; red arrow: strain curve of the SE module; green arrow: strain map

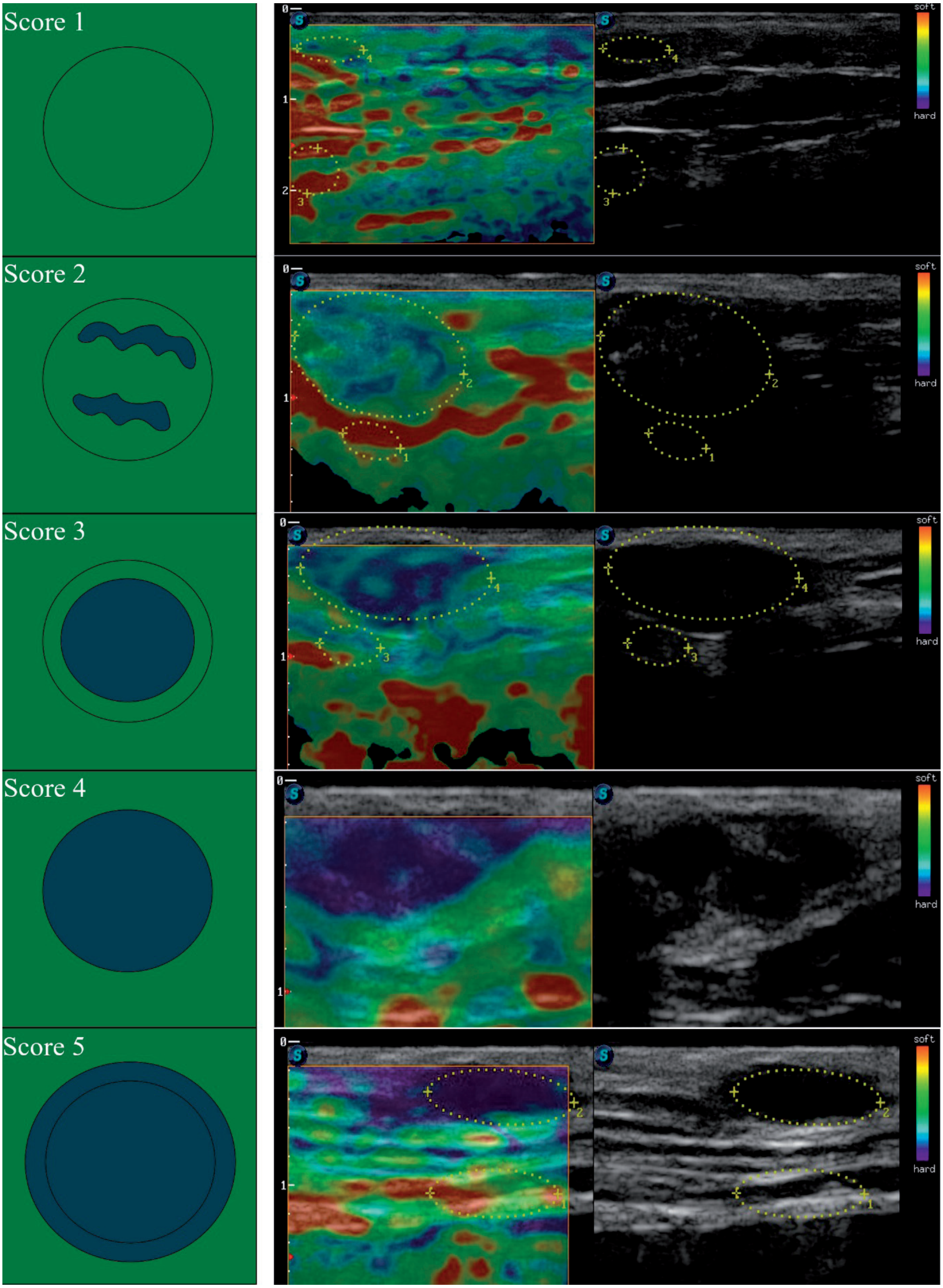

Fig. 2. Tsukuba elasticity scoring classification. Adapted from ITOH et al. (2006)

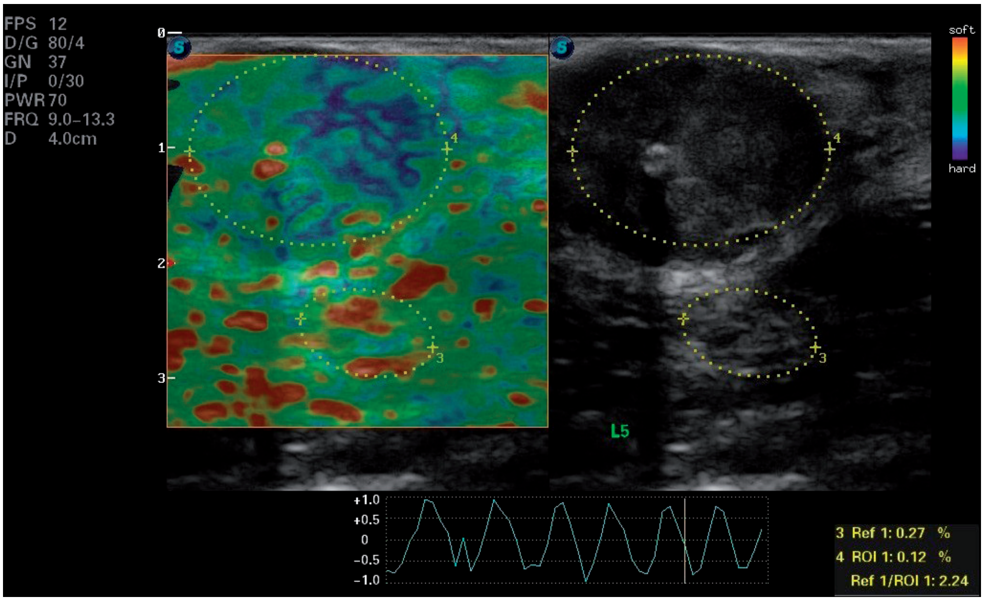

Fig. 3. Strain ratio measurement
Ref 1: strain value of the reference tissue; ROI 1: strain value of the lesion; Ref 1/ROI 1: strain ratio

The neoplasms were initially fixed in a 10% formaldehyde solution for 24 hours. Subsequently, routine laboratory procedures were carried out and they were embedded in paraffin blocks. They were cut by rotary microtome into 3-4 µm thick sections. The sections were dried, the paraffin removed and they were stained with hematoxylin and eosin. Finally, they were examined under a light microscope and classified histopathologically according to the criteria reported by GOLDSCHMIDT et al. (2011).

The SPSS 13.0 package program (SPSS Inc. Chicago, Illinois, USA) was used for the statistical analyses. The Shapiro-Wilk test was utilized in order to verify the normal distribution of the data. Since the results were not normally distributed, the Mann-Whitney U test was used for the statistical comparison of benign and malignant groups in terms of Tsukuba elasticity score classification and strain ratio. Values were given as mean ± standard error of the mean (SEM). The Likelihood Ratio test was used to determine the sensitivity and specificity values of the results to distinguish between the malignant and benign lesions. Receiver operator characteristics curve analysis was performed in order to determine the optimal cut-off value of the Tsukuba elasticity scores and the strain ratios.

The Kruskal-Wallis test was applied to compare malignant epithelial tumors in terms of Tsukuba elasticity score and strain ratio in different grade groups. The Mann-Whitney U test served to compare malignant epithelial tumor and malignant mixed tumor groups in terms of Tsukuba elasticity score and strain ratio. P values of less than 0.05 were considered to be statistically significant.

Results

Histopathology analysis revealed that 26.3% of the neoplasms were benign (n=15), and 73.7% were malignant (n=42). Further, 7.0% of these neoplasms were observed in cranio-thoracic mammary glands (n=4), 12.3% in caudo-thoracic mammary glands (n=7), 19.3% in cranio-abdominal mammary glands (n=11), 28.0% in caudo-abdominal mammary glands (n=16), and 33.4% in inguinal mammary glands (n=19).

The benign neoplasms consisted of adenosis (n=8), simple adenoma (n=3), benign mixed tumor (n=2), complex adenoma (n=1) and myoepithelioma (n=1). The malignant neoplasms in this study were complex type carcinoma (n=14), malignant mixed tumor (n=12), simple type tubulopapillary carcinoma (n=5), simple type tubular carcinoma (n=4), squamous cell carcinoma (n=2), submillary malignant transformation-oriented hyperplastic lesions (n=2), ductal carcinoma (n=1), a mixed type carcinoma (n=1) and a solid carcinoma (n=1).

Only 3 dogs had completely benign neoplasms, and 16 dogs had completely malignant tumors. Both benign and malignant neoplasms were found together in 4 dogs. Additionally, two or more neoplasms with similar or different histopathological features, located in the same or different mammary glands, were observed in 15 dogs.

In this study, the average value of the benign neoplasia elasticity scores was calculated as 1.66±0.15. The mean elasticity score of the malignant tumors was found to be 3.33±0.16. The elasticity scores of malignant tumors were significantly higher than the elasticity scores of benign neoplasms ($P<0.001$). According to these results, the optimal cutoff value was found to be between 2 and 3 (2.5), with values of 71.4% sensitivity and 93.3% specificity. The Tsukuba elasticity score distributions of mammary neoplasms are presented in Table 1.

In this study, the minimum and maximum strain ratios obtained from 15 benign neoplasms were 0.77 and 4.04, respectively. The mean strain ratio of all the benign neoplasms was calculated as 1.85±0.21. The minimum and maximum strain ratios obtained from the malignant tumors were measured as 1.56 and 7.57, respectively. The mean strain ratio of the malignant tumors was calculated as 3.76±0.23. The strain ratios of the malignant tumors were significantly higher than the strain ratios of the benign neoplasms ($P<0.001$). In the analysis of strain ratio measurements, the optimal cutoff value was found to be 2.15 with 90.5% sensitivity and 80.0% specificity.

The data obtained by comparing the Tsukuba elasticity scores and strain ratios of grade 1, 2 and 3 groups in the malignant epithelial tumors are presented in Table 2. The data obtained by comparing the Tsukuba scores and strain ratios of the malignant epithelial tumors and the malignant mixed tumor groups are classified in Table 3.

Table 1. Tsukuba elasticity score distributions of mammary neoplasms

	Score 1	Score 2	Score 3	Score 4	Score 5
Benign	n = 6	n = 8	n = 1	-	-
Malignant	-	n = 12	n = 12	n = 10	n = 8

Table 2. Tsukuba elasticity scores and strain ratios of grading groups in malignant epithelial tumors

	Mean	± SEM	Median	Minimum	Maximum	P value
Tsukuba score						0.600
Grade 1	3.18	0.40	3	2	5	
Grade 2	3.11	0.42	3	2	5	
Grade 3	3.50	0.27	3.5	2	5	
Strain ratio						0.571
Grade 1	3.73	0.48	3.22	2.07	7.57	
Grade 2	3.47	0.44	3.60	1.56	4.87	
Grade 3	4.41	0.62	3.87	2.00	7.39	

SEM: standard error of mean

Table 3. Tsukuba elasticity scores and strain ratios of two different histopathological groups in malignant mammary tumors

	Mean	± SEM	Median	Minimum	Maximum	P value
Tsukuba score						0.489
MET	3.27	0.21	3	2	5	
MMT	3.50	0.23	3.50	2	5	
Strain ratio						0.616
MET	3.88	0.30	3.41	1.56	7.57	
MMT	3.50	0.33	3.39	2.18	6.28	

SEM: standard error of mean; MET: malign epithelial tumor; MMT: malign mixed tumor

Discussion

Diagnosis and treatment of various neoplasms have an important place in clinical studies in veterinary medicine. Neoplasms of the mammary gland are the most common neoplasms in female dogs (SORENMO et al., 2011). In the literature, it was reported that the frequency of malignant mammary tumors is higher than benign ones (ATALAY VURAL and AYDIN, 2001; EŽERKYSTE et al., 2011; NUNES et al., 2019). The results of 26.3% benign and 73.7% malignant neoplasia obtained in this study also indicate that mammary tumors in female dogs were mostly malignant, which aligns with previous reports (ATALAY VURAL and AYDIN, 2001; EŽERKYSTE et al., 2011; NUNES et al., 2019).

The tissue volume of abdominal and inguinal mammary glands is higher than for thoracic mammary glands. Neoplasia occur more frequently in abdominal and inguinal mammary glands due to their increased tissue density (SORENMO et al., 2011). When the occurrence of mammary tumors was evaluated in relation to the anatomical location of the mammary glands, it was reported that neoplastic lesions were more frequently diagnosed in the abdominal and inguinal mammary glands (ATALAY VURAL and AYDIN, 2001; EŽERKYSTE et al., 2011; NUNES et al., 2019). In accordance with other research (ATALAY VURAL and AYDIN, 2001; EŽERKYSTE et al., 2011; NUNES et al., 2019), in this study neoplasia were mostly detected in the abdominal and inguinal mammary glands during B-mod USG scans. It has been reported that histopathologically different types of neoplasms can be found in the same or different mammary glands of a dog (ATALAY VURAL and AYDIN, 2001). In the present study, both benign and malignant tumors were diagnosed at the same time in different mammary glands in 4 dogs. In one dog, two different types of neoplasia were histopathologically observed in the same mammary gland. These findings were found to be compatible with ATALAY VURAL and AYDIN (2001).

The fact that breast glands in women contain enough adipose tissue and that compression can be applied means they constitute a practical and ideal environment for application of SE (KUMM and SZABUNIO, 2010). In female dogs, it has been reported that, while the adipose gland is concentrated in the inguinal mammary glands, it gradually decreases towards the thoracic glands (SORENMO et al., 2011). In addition to this information, it was observed that the thoracic cage under the thoracic mammary glands also made compressibility difficult. Due to these anatomical features, more repetitions are often required to apply the ideal pre-compression prior to SE measurements of the thoracic mammary glands. Therefore, SE application and measurements in the abdominal and inguinal glands were found to be easier and more practical for the practitioner.

ITOH et al. (2006), who developed the Tsukuba elasticity scoring, reported that 40 benign lesions showed score distributions of 1 and 2 in human breast cancer. YERLİ et al. (2011) indicated that no benign lesion was defined with an elasticity score of 5. In this study, the most benign neoplasia were classified as score 1 and score 2, and no malignant tumor was classified as score 1. In addition, none of the benign neoplasms were defined by elasticity scores of 4 and 5 in this study. Similar results were obtained in this study with the researchers who analyzed the elasticity scores with regard to the benignancy and malignancy of the tumors (ITOH et al., 2006; YERLİ et al., 2011). ITOH et al. (2006) reported that score 1 and 2 were to be considered as benign, score 3 as probably benign, and scores 4 and 5 as malignant in the Tsukuba elasticity score classification. In contrast to other researchers (ITOH et al., 2006), score 3 indicated malignancy in the present study. As in previous reports (ITOH et al., 2006), score 1 indicated benignity and score 2 indicated probable benignity in this study. However, the success rate of using the elasticity scoring classification to differentiate between malignant and benign mammary neoplasms ($P<0.001$) in the present study was found to be similar to the literature on human medicine (ITOH et al., 2006; KUMM and SZABUNIO, 2010; YERLİ et al., 2011; ZHAO et al., 2012; OKAR ATABEY et al., 2014).

When calculating the strain ratio in SE, an ultrasonographically normal site is required as a reference. The reference site for SE strain ratio measurements of breast neoplasms in humans is selected from ultrasonographically normal breast tissue at the same depth as the neoplastic lesion (KUMM and SZABUNIO, 2010; ZHAO et al., 2012). During the calculation of the strain ratio, it is recommended to choose a site close to the lesion and at the same depth for reference. However, this may not be possible in practice, and the practitioner may have to select a site with a different depth as the reference tissue (HOSKINS, 2011). For example, JEON et al. (2015) chose the abdominal wall for reference when evaluating the spleen, liver, kidney, and prostate gland in dogs. A similar study was performed in healthy cats, where the abdominal wall was taken as a reference (WHITE et al., 2014). Since there was not enough density of mammary gland in the thoracic mammary glands of the female dogs that could be considered as a reference, the abdominal and thoracic wall were used as reference tissues for strain ratio measurements. Although the species (humans and dogs) and reference sites were different, the success ratio ($P<0.001$) for using the strain ratios to distinguish between malignant and benign mammary tumors in this study was similar to reports in human medical literature (KUMM and SZABUNIO, 2010; YERLİ et al., 2011; ZHAO et al., 2012).

Histologically, the degree of malignancy in neoplasms was scored according to the tubule formation, nuclear pleomorphism, and mitosis ratio of the cells, and grouped into 1st, 2nd, and 3rd degrees from low malignancy to high (KARAYANNOPOULOU et al., 2005; GOLDSCHMIDT et al., 2011). In mammary tumors, the malignancy degree significantly affects the prognosis of the patient (KARAYANNOPOULOU et al., 2005; GOLDSCHMIDT et al., 2011). Therefore, in the present study, the ability of a non-invasive and rapid diagnostic tool such as SE to detect the degree of malignancy in neoplasms was evaluated. However, according to the data of this study, it was found that the Tsukuba elasticity scores and strain ratio results were not useful in distinguishing epithelial neoplasms according to their malignancy ($P>0.05$). PASTOR et al. (2022) reported that no association was found between the strain ratio and the histological grade of malignancy. In the present study, the strain ratio results of neoplasms that were obtained according to their malignancy was found to be similar to the results of the previous study (PASTOR et al., 2022).

Both malignant epithelial and malignant mesenchymal components are present in the histological structure of malignant mixed tumors (GOLDSCHMIDT et al., 2011). GOLDSCHMIDT et al. (2011) reported that malignant mixed tumors are rare neoplasms in the mammary glands. However, in the results of this study, it was observed that these types of neoplasms occupy a significant place (n=12) in all malignant neoplasms. On the basis of this information, Tsukuba elasticity scores and strain ratio results in malignant mixed tumors were compared with the results of malignant epithelial tumors. The fact that there was no significant difference between these two groups

(P>0.05) showed that the SE methods in this study were not successful in distinguishing these two malignant histological groups. It was suggested that insignificant results were obtained due to the malignancy in both groups.

Conclusions

Tsukuba elasticity score classification and strain ratio measurements can be made on the same elastograms in strain elastography. Through this study, it is thought that the strain elastography method will show better diagnostic capacity when the Tsukuba elasticity score classification, with 93.0% specificity, and the strain ratio methods, with 90.5% sensitivity, are used together. Strain elastography, which is an easy-to-apply technique, is thought to be highly beneficial as a non-invasive diagnostic tool in terms of regular follow-up in dogs with mammary neoplasia, which was previously evaluated histopathologically or cytologically, and for controlling lesions that are too small to be detected by palpation and other examination methods.

To the best of our knowledge, this is the first report that evaluates mammary tumors in female dogs with the two different SE measurement techniques in terms of benignancy and malignancy. Although increased Tsukuba elasticity scores and strain ratio were determined in malignant mammary tumors, these parameters were not successful in distinguishing tumor subtypes and histological grades. It was concluded that Tsukuba elasticity score classification and the strain ratio method were capable enough to distinct malignant mammary tumors from benign ones.

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Orcid ID

M. O. Şeran: https://orcid.org/0000-0001-6900-8332
Z. G. Uçmak: https://orcid.org/0000-0003-2530-1291
Ç. Tek: https://orcid.org/0000-0002-6429-658X

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SAŽETAK

Cilj je rada bio prosuditi dijagnostičku učinkovitost *strain*-elastografije u procjeni neoplazmi mliječne žlijezde. Istraživanjem su obuhvaćena 23 psa i ukupno 57 neoplazmi mliječne žlijezde. Na elastografskim zapisima izmjereni su faktor čvrstoće (engl. *elasticity score*) i omjer čvrstoće (engl. *strain ratio*) radi usporedbe histopatoloških nalaza kod neoplazmi mliječne žlijezde. Prosječna vrijednost faktora čvrstoće bila je 1,66±0,15 za dobroćudne neoplazme i 3,33±0,16 za zloćudne tumore (P<0,001). Optimalna granična vrijednost bila je između 2 i 3 (2,5), s osjetljivošću 71,4% i specifičnošću 93,3%. Prosječan omjer čvrstoće bio je 1,85±0,21 za dobroćudne neoplazme i 3,76±0,23 za zloćudne tumore (P<0,001). Analiza izmjerenih vrijednosti pokazala je da je optimalna granična vrijednost 2,15 s osjetljivošću 90,5% i specifičnošću 80,0%. Nije bilo statistički znakovite (P>0,05) razlike među tim dvjema metodama u analizi zloćudnih epitelnih tumora u različitim stadijima, a statistički znakovite (P>0,05) razlike nije bilo ni između zloćudnih mješovitih tumora i zloćudnih epitelnih tumora. Rezultati ovog istraživanja pokazali su da je *strain*-elastografija izvrstan alat u diferencijalnoj dijagnostici neoplazmi mliječne žlijezde kuja.

Ključne riječi: tsukuba faktor čvrstoće; elastografija; omjer čvrstoće; tumor mliječne žlijezde; pas