

Diagnostic Value of Ultrasound Elastography for Characterization of Solid Breast Lesions

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ABSTRACT

Objective: In this study, the contribution of ultrasound (US) elastography in the characterization of solid breast masses was investigated.

Methods: Seventy-five patients (1 male and 74 female, aged between 19 and 80 years) were enrolled. A total of 75 solid breast lesions, for which a biopsy was planned, were evaluated using B-mode US and US elastography during the same session. Using B-mode US, the lesions were classified according to the Breast Imaging Reporting and Data System (*BI-RADS*); Tsukuba elasticity score was determined and strain ratio was calculated for all lesions. Subsequently, a core biopsy of the lesions was performed. *BI-RADS* results, Tsukuba elasticity scores, and strain ratio were compared with the histopathological results.

Results: Of 75 lesions, 47 were benign (62.66%) and 28 were malignant (37.33%). According to the Tsukuba scoring method, the mean score of benign and malignant lesions was 2.31 and 3.96, respectively. The mean strain ratio was calculated to be 4.97 ± 2.94 (0.96–13.20) for malignant lesions and 2.27 ± 1.41 (0.5–5.84) for benign lesions. In statistical analysis, the mean strain ratio of the malignant lesions was significantly higher than that of the benign lesions (p<0.05). *BI-RADS* classification had the highest sensitivity (89.3%), and the Tsukuba scoring method had the highest specificity (93.6%). Elastographic assessment altered the result in 6 of 8 patients (75%) that B-mode US was non-diagnostic

Conclusion: The combination of B-mode US and US elastography can significantly improve the accuracy of diagnosis and characterization of breast lesions, thereby reducing the unnecessary biopsy rate.

Keywords: BI-RADS, elastography, solid breast lesions, ultrasound

INTRODUCTION

Breast cancer is the most common malignant neoplasm in women both in the world and in Turkey, and it constitutes approximately 30% of all cancers detected in women and about 20% of cancer-related deaths (1, 2). The incidence is high in developed countries and is also increasing in the underdeveloped countries. The most common cancer-related deaths are in Europe, and the second most common cause of death is breast cancer in the United States (3). In the United States and in the Western European countries, one in every eight women is at risk of breast cancer throughout life (4, 5).

Although mammography and ultrasonography (US) are frequently used and there are valuable imaging methods to reveal palpable or non-palpable breast lesions, the number of unnecessary biopsies may increase due to the confusion in distinguishing benign solid lesions from malignant ones. This provides the basis for the development of non-invasive imaging modalities that will contribute to the differentiation of benign and malignant lesions. US elastography, which is one of these methods, is increasingly used in the differentiation between benign and malignant lesions both in the breast and in many organs and for different clinical indications.

Elastography is a US technique that reflects the stiffness of the lesion, simultaneously produces the tension map

of the tissues subjected to compression, and qualitatively and quantitatively reveals this information (6, 7). Malignant lesions are less deformed under pressure than are normal tissue and benign lesions. Elasticity maps and scores generated based on this principle give good results both in the differentiation of normal–abnormal tissue and benign–malignant lesion (7).

The aim of this study was to determine the contribution of US elastography to lesion characterization by comparing the histopathological results with conventional US and US elastography findings in solid breast lesions requiring biopsy.

METHODS

In this study, a total of 75 patients, 74 females and one male, who applied to the Mersin University Medical Faculty Center for Health Research and Practice between January 2015 and December 2015 and in whom a mass was detected radiologically and through examination in the breast were evaluated retrospectively. The mean age was 48±13 years (19-80). Before inclusion, all the patients were informed regarding the study and the procedures to be conducted, and informed consent was obtained from the patients. This study was approved by Mersin University Institutional Review Board (Project no: 2015/195). Patients who were under 18 years of age, who were thought that biopsy was not required as a result of clinical-radiological

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evaluation despite presence of solid breast lesion, who had a localized systemic disease that prevented biopsy, and who previously underwent biopsy and received histopathological diagnosis were excluded from the study.

There were 75 lesions in 75 cases. Lesions were examined simultaneously using the B-mode US and strain elastography before the biopsy. All the examinations were performed by the same radiologist using the same device (Toshiba Aplio 500, Toshiba Medical System Co., Tokyo, Japan) with a real-time elastography software and a transducer (14 MHz linear transducer).

After the demographic information of the cases was recorded, the patients were positioned according to the location of lesions. Firstly, the B-mode US was performed. Lesions were centralized and the localization, size, shape, border, orientation, internal echogenicity, internal structure (solid/cystic), calcification, posterior acoustic features, and surrounding tissue of each lesion were assessed perpendicularly to the skin, lesion, and chest wall. In the light of this evaluation, the lesions were classified according to the Breast Imaging-Reporting and Data system (BI-RADS). According to this, category 2 lesions were evaluated as benign; category 3 lesions as most likely benign; category 4 lesions as low suspicion for malignancy; and category 5 lesions as highly suspicious for malignancy.

Subsequently, the elastography mode was switched on. The screen of the device was divided into two parts: one for the Bmode image and the other for elastography. The imaging field was set to include the entire mass, the subcutaneous fat layer, and the superficial layer of the pectoral muscle. When elastography images were obtained, mild rhythmic manual pressure was applied perpendicularly to the lesion. To confirm that the sonoelastographic images were obtained with proper compression and while a mild pressure was applied on the skin using the transducer, the quality factor on the US instrument screen was provided to be ≥55, which is the standard value of the breast elastography for Toshiba Aplio 500. During the procedure, the motion amplitude of the transducer in the vertical direction was 1-2 mm, and the average motion velocity was 1-2 per second. For each pixel of the elasticity image, one of 256 specific colors was observed according to the strain degree. The color scale changed from red in soft tissues, where the strain was the highest (the softest), to the blue in hard tissues, where there was no strain (the hardest); the green color showed the average strain. A five-point Tsukuba scoring method developed by Itoh et al was used for the evaluation of elastography images. According to this, predominantly, the green-coded lesions having equal elasticity with the surrounding mammary parenchyma were evaluated as score 1; lesions having inhomogeneous elasticity and blue-green areas were evaluated as score 2; lesions that were coded green at the periphery and blue at the center were evaluated as score 3; bluecoded lesions were evaluated as score 4; and blue-coded lesions that have an echogenic halo around and whose surrounding tissue lost elasticity were evaluated as score 5. The lesion score of 1-3 was considered benign, and the score 4-5 was considered malignant.

After the elastograms were obtained, the strain values of the mass and the surrounding fat tissue was numerically measured

through the static image using ROI and was automatically proportioned by the device. In the measurements of mass and fat tissue, the maximum depth difference was set to be 5 mm. This strain ratio (SR) obtained by dividing the fat tissue strain value by the mass strain value (strain index [SI]) was measured and recorded twice for each mass.

After the US and sonoelastographic examination was completed, biopsies were performed in the evaluated lesions using a cutting-needle under US guidance. Biopsies of two pieces of length at least 15 mm or 22 mm from each lesion was taken using an automatic pistol and a 14 G biopsy needle compatible with it. Histopathology results of the materials were recorded.

Statistical Analysis

For the statistical analysis, the computer program Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) 21.0 for Windows 7 was used. Descriptive values of the data obtained in the study were given as the number of patients, mean, standard deviation, and maximum and minimum values. An independent Student-t test was used in evaluating the differences in the average values of the parameters between the independent groups, and receiver operating characteristics (ROC) curve was used to evaluate the performances of the diagnostic tests. A p value of <0.05 value was considered statistically significant.

RESULTS

The mean age of 75 patients (1 male and 74 female) included in the study was 48.13 years. Based on the histopathological evaluation, 47 (62.66%) lesions were reported as benign and 28 (37.33%) as malignant. The distribution of lesions according to histopathological results is shown in Tables 1 and 2.

The mean age of the patients diagnosed with benign lesions was 44 ± 12 years and of those diagnosed with malignant lesions was 54 ± 13 years. The maximum mean size of all lesions was 18 ± 10 mm, of the malignant lesions was 22 ± 11 mm, and of the benign lesions was 24 ± 19 mm. The data of patients' age, histopathological diagnosis of the lesions, and the largest lesion size are shown in Table 3.

The distribution of benign and malignant lesions according to the BI-RADS scoring system is shown in Table 4.

While all 16 lesions evaluated as BI-RADS 5 according to the B-mode US were histopathologically diagnosed as malignant lesions; only 1 lesion evaluated as BI-RADS 3 was identified as malignant. When BI-RADS 3 and 4a lesions were considered benign and BI-RADS 4b, 4c, and 5 lesions were accepted as malignant, the sensitivity of the Bmode scoring method was 89.3%, specificity was 89.4%, accuracy was 89.3%, positive predictive value was 93.3%, and negative predictive value was 83.3%.

In the sonoelastography method in which the five-point scoring method was used, the mean scores of benign and malignant lesions were calculated to be 2.31 and 3.96, respectively, (Figure 1, 2). The distribution of benign and malignant lesions according to their elasticity scores is shown in Table 5. When the scores 1–3 were accepted as benign and the scores 4 and 5 as malignant, the sensitivity of the five-point scoring method was 75%, specificity was 93.6%, accuracy was 86.7%, positive

Table 1. Lesions diagnosed histopathologically as benign

Diagnosis	Number	%
Fibroadenoma	20	26.7
Fibrosis	9	12.0
Fibroadipose tissue	3	4.0
Fat necrosis	3	4.0
Granulomatous mastitis	3	4.0
Ductal hyperplasia	2	2.7
Fibrocystic change	2	2.7
Intracutaneous papillary lesion	2	2.7
Lactational adenoma	1	1.3
Benign fibroepithelial lesion	1	1.3
Mastitis	1	1.3
TOTAL	47	62.66

Table 2. Lesions diagnosed histopathologically as malignant

Diagnosis	Number	%
Invasive ductal cancer	24	32
Ductal carcinoma in situ	3	4
Solid papillary cancer	1	1.33
TOTAL	28	37.33

Table 3. Age of the patients, histopathologic diagnosis of lesions, and mean lesion size

Pathology	Number	Average Age	Average Size
Benign	47	44±12	16±9 mm
Malignant	28	54±13	22±11 mm
TOTAL	75	48±13	18±10 mm

Table 4. According to histopathological diagnosis, the distribution of lesions which were classified in reference to ultrasonographic BI-RADS terminology

US-BI-RADS	Benign	Malignant	Total
3	28 (37.3%)	1 (1.3%)	29 (38.3%)
4a	14 (18.7%)	2 (2.7%)	16 (21.3)
4b	5 (6.7%)	1 (1.3%)	6 (8%)
4c	0	8 (10.7%)	8 (10. 7%)
5	0	16 (21.3%)	16 (21.3%)
TOTAL	47 (62.7%)	28 (37.3%)	75 (100%)

Table 5. Distribution of benign and malignant lesions according to their elasticity scores

Elasticity Score	Benign	Malignant	Total	
1	5 (6.7%)	0	5 (6.7%)	
2	25 (33.3%)	2 (2.7%)	27 (36%)	
3	14 (18.7)	5 (6.7%)	19 (25.3%)	
4	3 (4%)	13 (17.3%)	16 (21.3%)	
5	0	8 (10.7%)	8 (10.7%)	
TOTAL	47 (62.7%)	28 (37.3%)	75 (100%)	

predictive value was 87.5%, and negative predictive value as 86.3%. According to the five-point scoring method, there were three false-positive lesions (2 fibroadenomas and 1 intraductal papillary lesions) and seven false-negative lesions (4 intraductal carcinomas, 2 ductal carcinomas in situ, and 1 solid papillary carcinoma; Figure 3).

The average SR was 4.97±2.94 (0.96–13.20) for the malignant lesions and 2.27 ± 1.41 (0.5–5.84) for the benign lesions. The mean SR of the malignant lesions showed higher statistically significant value than that of the benign lesions (p<0.05). In the ROC analysis performed for the SR, when the cut-off value for the differentiation of benign-malignant lesions was selected as 3.09, the accuracy of the method was 77.3%, sensitivity was 71.4%, specificity was 80.9%, positive predictive value was 69%, and negative predictive value was 84.4%. According to the SR method, nine false-positive lesions (7 fibroadenomas and 2 fibrosis) and seven false-negative lesions (6 invasive ductal carcinomas and 1 ductal carcinoma in situ) were detected. The comparison of the performances of the B-mode US and the sonoelastographic five-point scoring and the SR method in the differentiation between benign and malignant breast masses is presented in Table 6.

When the diagnostic performance values of the B-mode US, the sonoelastographic five-point scoring system, and the SR methods were compared, the sensitivity of the B-mode US was higher than both five-point scoring system and SR method; while the specificity was lower than the five-point scoring system, it was higher than the SR method. The method with the highest sensitivity was the B-mode US and the method with the highest specificity was the five-point scoring system.

Three lesions defined as BI-RADS 3 or 4a in the B-mode US and predicted as benign but diagnosed as malignant lesion in the histopathological examination, and five lesions defined as BI-RAD 4b in the B-mode US and predicted as malignant lesion but diagnosed as benign lesion in the histopathological examination were evaluated using the five-point scoring system and the SR method.

One lesion (ductal carcinoma in situ) identified as BI-RADS 3 in the B-mode US but histopathologically diagnosed as malignant was defined as benign in the five-point scoring system and SR method.

Table 6. Comparison of the performances of sonoelastographic five-point score and strain ratio methods and B-mode US for the differentiation between benign and malignant breast masses

Method	Accuracy	Sensitivity	Specificity	Negative Prediction Value	Positive Prediction Value
B-mode US	89.3%	89.3%	89.4%	83.3%	93.3%
Five-point scoring	86.7%	75%	93.6%	86.3%	87.5%
Strain index	77.3%	71.4%	80.9%	84.4%	69%



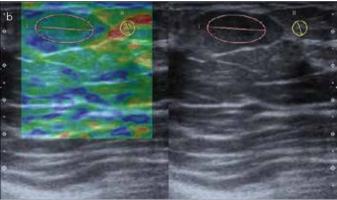


Figure 1. a, b. A 43-year-old female patient. (a) Lesion of BI-RADS category 3. (b) Tsukuba score 3 on US elastography and strain ratio of 2,3. Histopathological diagnosis: Fibroadenoma

One lesion (invasive ductal carcinoma) identified as BI-RADS 4a according to the B-mode US but histopathologically diagnosed as malignant was defined as malignant according to the five-point scoring system and benign according to the SR method. One lesion (ductal carcinoma in situ) accepted as BI-RADS 4a and whose histopathological result was malignant was interpreted as benign according to the five-point scoring system and as malignant according to the SR method.

Four of the five lesions defined as BI-RADS 4b according to the B-mode US and diagnosed histopathologically as benign (2 granulomatous mastitis, 1 ductal hyperplasia, and 1 fibroadenoma) were defined as benign according to the fivepoint scoring system, and three of them (2 granulomatous mastitis, 1 ductal



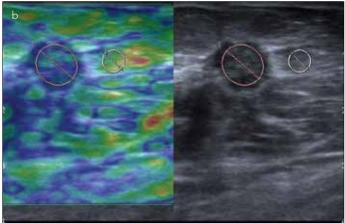


Figure 2. a, b. A 68-year-old female patient. (a) Lesion of BI-RADS category 5. (b) Tsukuba score 5 on US elastography and strain ratio of 6,82. Histopathological diagnosis: Invasive ductal carcinoma

hyperplasia) were defined as benign according to the SR method (Figure 4).

Thus, in six of the eight patients (75%) in whom the B-mode US could not reach an accurate result, the elastographic US method (five-point scoring system or SR method) was found to change the result in the correct direction.

The results of the lesion distribution in the BI-RADS-five-point scoring system and the BI-RADS-SR method are shown together in Figure 5 and 6.

When the diagnostic performance of the five-point scoring method and the SR method were compared, three lesions that were found to be false-negative according to the five-point scale were

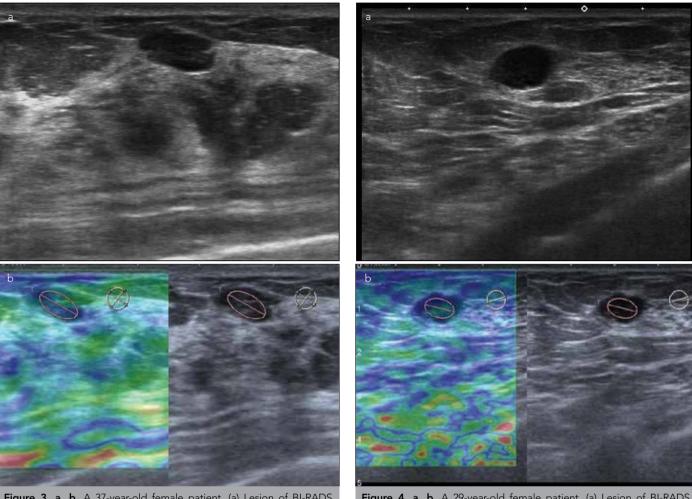
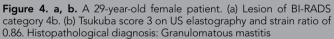
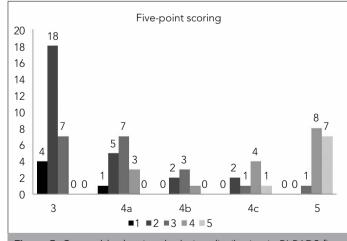


Figure 3. a, b. A 37-year-old female patient. (a) Lesion of BI-RADS category 3. (b) Tsukuba score 3 on US elastography and strain ratio of 2.03. Histopathological diagnosis: Ductal carcinoma in situ





 $\begin{tabular}{ll} \textbf{Figure 5.} Cross-table showing the lesion distribution in BI-RADS-five-point scoring system \\ \end{tabular}$

malignant according to the lesion SR method. One lesion that was false-positive according to the five-point scoring method was benign according to the SR method. When the ROC curves of both methods were plotted, the area under the curve was

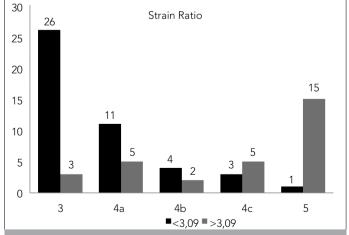


Figure 6. Cross-table showing the lesion distribution in BI-RADS-strain ratio method

found to be 0.902 for the five-point scoring and 0.809 for the SR method. When the performance values of the two methods were compared, the five-point scoring method was found to be superior to the SR method.

DISCUSSION

Breast cancer is a type of cancer that ranks on the top (18%) of cancer-related deaths in women all over the world and has a lifetime risk of development with a rate of 7%-10% (1). Since it is a very common tumor, many studies have been conducted to investigate the early diagnosis and optimal treatment of breast cancer. The main purpose of imaging methods, which is one of the most important aspects of diagnosis, is to detect breast cancer at an early stage by maximally avoiding unnecessary invasive procedures. The routine practice is to provide a histopathological diagnosis through biopsy in the lesions, which are determined by physical examination and radiological evaluation and are suspected to be malignant. On an average, 30%-60% of the biopsies performed in patients who do not carry all the malignant findings but in whom malignancy cannot be excluded (BI-RADS 4 lesions) are diagnosed histopathologically as benign lesions. The common goal in radiological examinations performed during breast imaging is to increase the sensitivity in detecting malignancy as well as specificity (8, 9).

In recent years, the US elastography method, which is being increasingly used in evaluating soft tissue lesions, is also a candidate that plays an active role in the characterization of breast lesions. Studies have shown that US elastography has a sensitivity of 78%–100% and a specificity of 21%–99% in distinguishing solid breast lesions as malignant or benign. An important limitation of the method is the variability among the evaluators. To eliminate this limitation, studies have been conducted using more objective and fixed criteria such as those in the SR method. These studies have shown that elastography can be a good complementary test with high sensitivity and specificity (10).

In our study, the sensitivity of the B-mode US was 89.3% and specificity was 89.4%. The five-point scoring method named "Tsukuba" defined by Itoh et al. (11) was used in the elastographic classification, and the sensitivity of the five-point scoring method was found as 75% and the specificity as 93.6%. In the study of Itoh et al. (11), which involved 111 lesions, the sensitivity of the five-point scoring method was reported as 86.5% and the specificity as 89.8%. In a 139-lesion investigation by Zhu et al. (12), the sensitivity was 85.5% and the specificity was 86.6%. The results of our study were consistent with the previous studies using the five-point scoring method and demonstrated that this method could increase the specificity in breast masses.

The main problem with the five-point scoring method is that the evaluation is subjective due to the diversity of the images, and there may be discordance among the observers. More objective measurement methods were needed to overcome this situation (13, 14). The SR method, which is a semi-quantitative US elastography method, has been suggested for this purpose. The rate of strain is the ratio of the examined tissue strain value to the tissue strain value of the adjacent normal glandular texture or the subcutaneous fat tissue (13-15). The selection of the reference point in the measurement of the SR is important in terms of the accuracy of the method. It has been suggested that the reference point should be selected from the subcutaneous fat tissue at the same depth for the accurate measurement of the SR in the differentiation of benign and malignant breast lesions.

In this study, the subcutaneous fat tissue at the same depth as the lesion was taken as a reference in the measurement of the SR, and the average value of the SR was calculated as 4.97±2.94 (0.96-13.20) for malignant lesions and 2.27±41 (0.5-5.84) for benign lesions. The mean SR in malignant lesions showed a higher statistically significant value than that of the benign lesions (p<0.05). In the ROC analysis performed for the SR, when the cut-off value for the differentiation of benign-malignant lesion was taken as 3.09, the sensitivity of the method was 71.4% and the specificity was 80.9%. Different cutoff values have been reported in literature for the SR method. In a 559-patient (415 benign, 144 malignant) study by Zhi et al. (13) and in 187-patient (130 benign, 57 malignant) study by Zhao et al. (16), the best cut-off values were respectively found to be 3.05 and 3.06, and these values are close to 3.09 found in our study. In the study by Zhi et al. (13), the sensitivity was reported as 90% and the specificity as 89%; in the study by Zhao et al. (16), the sensitivity was reported as 84.2% and the specificity as 84.6%.

In our study, the diagnostic performance values of the SR and the five-point scoring method were also compared and the diagnostic performance of the five-point scoring method was found to be higher than that of the SR method. Studies have suggested that SR measurement is superior to the five-point scoring method, and there are also studies reporting that there is no significant difference between the diagnostic performances of the two methods. In a study that analyzed 559 lesions, Zhi et al. (13) found the sensitivity of the SI method as 92.4% and the specificity as 91.1%, and they found the diagnostic performance of this method higher than that of the five-point scoring. In a study that evaluated 227 breast lesions and the subcutaneous fat tissue at the same depth was taken as a reference in the SR measurement, Thomas et al. (17) determined the sensitivity of the SR method as 90% and the specificity as 89%. They found the diagnostic performance of the SR measurement higher than the five-point scoring method. In a 78-lesion study, Yerli et al. (18) made the SI measurement by considering the glandular tissue at the same depth as a reference and found the specificity of the SR as 93% and the sensitivity as 80%. They found the specificity of the scoring method as 95% and the sensitivity as 80% and reported that there was no statistically significant difference between the diagnostic performances of the SR and the five-point scoring method. Moreover, it was stated that the use of the SR measurement together with the scoring method would not provide an additional contribution. In our study, while the sensitivity of the B-mode US was found to be higher than those of the sonoelastographic five-point scoring and SI methods and the specificity was found to be lower than the five-point scoring method, it was found to be higher than the SR method. In six of the eight lesions in which the B-mode US evaluation and histopathological examination were inconsistent, the fivepoint scoring method and/or SI method were observed to eliminate the inconsistency and affected the radiological evaluation in the correct way. The histopathological diagnoses of these six lesions are granulomatous mastitis (2 lesions), ductal hyperplasia, fibroadenoma, ductal carcinoma in situ, and invasive ductal cancer.

Granulomatous mastitis is clinically and radiologically difficult to be differentiated from breast cancer. One of the three granulomatous mastitis cases in our study was evaluated as BI-RADS 4a and 2 of them as BI-RADS 4b. Particularly, the lesions classified as BI-RADS 4b could not be differentiated from the malignant lesions because they had indefinite boundaries and were irregularly shaped. However, these lesions were defined as benign in both the five-point scoring and the SR methods. This result supports that US elastography may be an effective method for differentiating granulomatous mastitis from malignant lesions. In a study by Durur-Karakaya et al. (19), 27 cases of granulomatous mastitis were retrospectively evaluated, and the average elastography score of the lesions was 1.66±0.55 and the average value of the SR was 1.10±0.79. It was concluded that granulomatous mastitis had a benign characteristic in US elastography; however, it should be supported by a larger series of prospective studies.

In our study, the histopathological diagnosis was fibroadenoma in two of three false-positive lesions in five-point scoring method and in seven of nine false-positive lesions in the SR method. Although they are defined with the same name, the fact that fibroadenomas are a heterogeneous lesion group and their histopathological internal structures differ may be the explanation of this error. Fleury et al. (20) reported that 115 fibroadenomas in their study included high strain parameters in US elastography due to the differences in internal structure, myxoid–mucinous content, high cellularity, and stromal fibrosis.

In our study, while four of the seven false-negative lesions were invasive ductal carcinoma and two were ductal carcinoma in situ according to the five-point scoring method, six of the seven false-negative lesions were invasive ductal carcinoma and one was ductal carcinoma in situ according to the SR method. In an 84-case prospective study by Fleury et al., it has been reported that breast cancer is presented in a wide spectrum in the US elastography and that the strain degrees of the different subgroups may change leading to false-negative results. In a retrospective study by Grajo and Barr (22), 266 malignant breast lesions were examined, and it was thought that low-grade invasive ductal carcinomas, mucinous cancers, ductal carcinoma in situ, and atypical ductal hyperplasia may cause false-negative results by including low strain degrees.

There are some restrictions in our study. US elastography is a method that requires experience and practice and is user-dependent similar to all US examinations. User dependence may have affected the results. The fact that the number of cases is low compared to the studies in literature and the absence of histopathological variability especially in malignant lesions can be considered as a restriction. In addition, the fact that there may be differences between the measurements made from the peripheral stroma and the lesion itself and that the measurement is not calculated separately from the peripheral stroma and the mass itself is another limitation.

CONCLUSION

In the characterization of solid breast lesions, the B-mode US has the highest accuracy. When the B-mode US, five-point scoring method, and SR method are compared, the B-mode US has the highest sensitivity, and the five-point scoring method has the

highest specificity. US elastography is a method that contributes to the B-mode US in the differentiation of benign-malignant solid breast lesions. In particular, in the BI-RADS 4 lesions that are suspicious in terms of malignancy, the presence of the SR method and/or the five-point scoring method may prevent unnecessary biopsies by reducing the number of false-positive and false-negative lesions and increasing the specificity.

Ethics Committee Approval: This study was approved by Mersin University Institutional Review Board (Project no: 2015/195).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.F.T.; Design – F.D.A.; Supervision – F.D.A., T.A.; Resources – M.F.T., T.K.; Materials – T.K.; Data Collection and/or Processing – S.T., K.E.; Analysis and/or Interpretation – S.T., Y.B.; Literature Search – Y.B.; Writing Manuscript – M.F.T., S.T.; Critical Review – F.D.A., K.E.

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