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Research Article

Predicting Breast Cancer Biomarker Expression with Diffusion-Weighted Magnetic Resonance Imaging

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ABSTRACT

Objective: To evaluate the diagnostic performance of diffusion-weighted magnetic resonance imaging (DWI) and apparent diffusion coefficient (ADC) values in differentiating benign and malignant breast lesions, and to investigate correlations between ADC values and immunohistochemical (IHC) biomarkers (estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, Ki-67) as well as axillary lymph node status.

Methods: This retrospective study included 148 female patients (159 breast lesions) who underwent preoperative breast magnetic resonance imaging (MRI) between January 2022 and December 2024. DWI was performed using b values of 0 and 1000 s/mm^2, and mean ADC values were calculated from regions of interest placed within solid tumor areas. Histopathological and IHC analyses were used to classify lesions and molecular subtypes.

Results: Among the 159 lesions, 56 (35.2%) were malignant and 103 (64.8%) were benign. Malignant lesions exhibited significantly lower ADC values than benign ones (0.92×10^{-3} mm^2/s vs. 1.66×10^{-3} mm^2/s). The optimal ADC cut-off for malignancy was 1.24×10^{-3} mm^2/s, yielding 95.2% sensitivity and 89.1% specificity. Hormone receptor-positive tumors and lesions with high Ki-67 index showed lower ADC values. No significant correlation was found between ADC and human epidermal growth factor receptor 2 status. Triple-negative breast cancers demonstrated the highest ADC values among subtypes. ADC values of metastatic axillary lymph nodes were significantly lower than those of contralateral benign nodes (0.78×10^{-3} vs. 1.82×10^{-3} mm^2/s).

Conclusion: ADC values are effective in distinguishing malignant from benign breast lesions and provide non-invasive insights into tumor biology. Lower ADC values correlate with malignancy, hormone receptor positivity, proliferative activity, and metastatic nodal involvement. DWI is a reliable, non-contrast imaging biomarker that may enhance personalized evaluation of breast cancer.

Keywords: Apparent diffusion coefficient values, estrogen receptor, progesterone receptor, immunohistochemical biomarkers, lymph node metastasis

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide and remains a leading cause of mortality. Accurate, non-invasive characterization of tumor biology is essential for early diagnosis and tailored treatment strategies. Magnetic resonance imaging (MRI), especially dynamic contrast-enhanced MRI, offers high sensitivity in lesion detection. However, due to the limitations associated with contrast agent use—such as nephrotoxicity and gadolinium retention—alternative, contrast-free imaging techniques have gained prominence. Among these, diffusion-weighted imaging (DWI) and the derived apparent diffusion coefficient (ADC) have emerged as promising tools for functional lesion characterization.[1,2]

DWI reflects the mobility of water molecules within tissues and provides indirect information about cellular density, stromal composition, and membrane integrity. Moreover,

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the quantitative evaluation of water diffusion is performed using ADC values derived from DWI. The ADC is expressed in square millimeters per second (mm^2/s) and is determined by quantifying signal attenuation on DWI acquired with at least two different b values. Malignant breast tumors typically demonstrate restricted diffusion and thus lower ADC values due to high cellularity, while benign lesions tend to exhibit higher ADC values.[3-6]

In addition to lesion characterization, DWI is increasingly investigated for its potential to reflect tumor molecular characteristics. Several studies suggest that ADC values may correlate with immunohistochemical (IHC) biomarkers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67, which are used to define tumor subtypes and aggressiveness.[7,8]

Moreover, axillary lymph node involvement, a key component in breast cancer staging and management, is also evaluated using DWI. By assessing the diffusion properties of lymph nodes, DWI may help distinguish between benign and metastatic nodes non-invasively.[9]

This study aims to comprehensively evaluate the diagnostic value of DWI-derived ADC measurements in differentiating benign and malignant breast lesions, to delineate their associations with major IHC biomarkers (ER, PR, HER2, Ki-67), and to determine their added utility in axillary staging through comparison of ADC values between metastatic ipsilateral and benign contralateral lymph nodes.

MATERIAL AND METHODS

Ethical Aspects

The study received ethical approval from the Clinical Research Ethics Committee at Erzincan Binali Yıldırım University (protocol number: EBYU-KAEK-2024-17-008-412224, date: 05.12.2024). All patients have provided written informed consent for their data to be included in the MRI studies database and used in scientific research.

Patient Selection

Patients who received breast MRI scans at our center from January 2022 to December 2024 were analyzed retrospectively. The inclusion criteria for our study were patients with masses identified in breast MRI for whom histological (obtained by needle biopsy and/or surgical intervention) sampling data were accessible, patients whose breast MRI reports were categorized as Breast Imaging Reporting and Data System (BI-RADS) 4/5/6, and patients with diagnostic-quality DWI and ADC images in breast MRI scans. Precautions were taken to ensure an interval of more than three weeks between the breast biopsy and the subsequent breast MRI scans.

Patients who did not have breast MRI before surgical excision were excluded from the study to avoid misinterpretations due to bleeding or postoperative changes. Patients for whom the results of the IHC biomarker and the histological examination could not be obtained were also excluded from the study. Furthermore, patients exhibiting only non-mass-like contrast enhancement on breast MRI and those for whom the lesion was undetectable on DWI images because of small lesion size or suboptimal image resolution were excluded from the study.

During the retrospective review of the targeted time frame, we identified 680 breast MRI scans performed at our center. Among these, 322 had breast lesions categorized as BI-RADS 4, 5, or 6. Seven patients with a history of breast surgery, 71 patients without histopathology and biomarker results, 67 patients exhibiting non-mass contrast enhancement, and 29 patients for whom quantitative analysis on DWI images was unfeasible were excluded from the study. Finally, this study included 148 female patients (Figure 1).

MAIN POINTS

- Apparent diffusion coefficient (ADC) values serve as a reliable non-invasive biomarker capable of precisely differentiating between benign and malignant breast lesions.
- Lower ADC values are substantially correlated with malignancy, hormone receptor positivity, and a high Ki-67 proliferation index.
- Metastatic axillary lymph nodes exhibit significantly reduced ADC values compared to benign nodes, underscoring the utility of diffusion-weighted imaging in axillary staging.

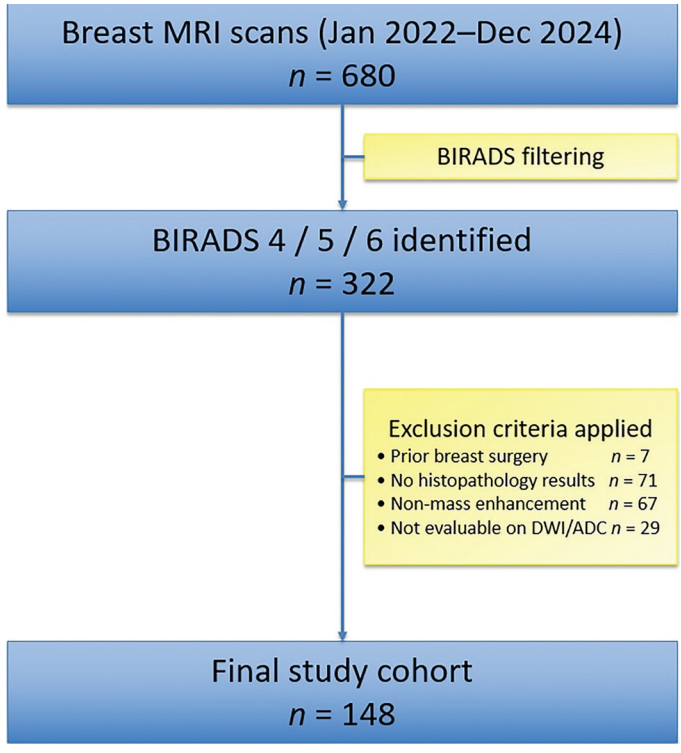

Figure 1. A flow chart illustrating the designation of the study cohort.

MRI, magnetic resonance imaging; BI-RADS, Breast Imaging Reporting and Data System. DWI, diffusion-weighted imaging; ADC, apparent diffusion coefficient

MRI Acquisition Protocol

A 1.5-T MRI scanner (Siemens Magnetom Aera, Erlangen, Germany) equipped with a dedicated 16-channel bilateral breast coil was used for all breast MRI examinations. Patients were positioned prone during imaging. The standardized protocol included 3D T1-weighted gradient-recalled echo sequences [repetition time (TR)/echo time (TE) = 20/4.5 ms; slice thickness = 3 mm] and T2-weighted fast spin-echo sequences (TR/TE = 4000/90 ms; slice thickness = 3 mm). For additional lesion characterization, an short tau inversion recovery sequence was acquired. DWI was performed using a single-shot echo-planar imaging technique with b-values of 0 and 1000 s/mm^2. The parameters for DWI included TR/TE = 5600/70 ms, field of view = 340 mm, slice thickness = 4 mm, and a matrix size of 128 × 128. ADC maps were automatically generated by the workstation software. All examinations adhered to a standardized institutional protocol.

Image Analysis

Image analysis was performed on a dedicated workstation (Syngo.via, Siemens Healthineers, Erlangen, Germany). The breast MRI scans of the study participants were evaluated by two radiologists. Observer A, with 10 years of experience, and Observer B, with 3 years of experience, jointly evaluated the MRI data sets without knowledge of the clinical and histopathological findings. Any disagreements among them were resolved by consensus. The recorded parameters included lesion size, morphological features (shape, margin, and internal architecture) according to the BI-RADS MRI lexicon, and DWI-signal intensity and ADC values obtained by placing a region of interest (ROI) within the solid portions of the lesion, carefully avoiding necrotic or cystic areas. At least three ROIs were placed, and the average ADC value was recorded. Additionally, ADC values of the most suspicious axillary lymph node on the ipsilateral side were measured in its cortical region. In these patients, the ADC value of the largest lymph node on the contralateral side was also recorded.

Histopathological and IHC Analysis

Histopathological evaluation was performed on tissue specimens obtained after biopsy or surgical resection, and tumors were classified as benign or malignant according to the World Health Organization criteria. IHC data analysis included assessment of ER and PR, with positivity defined as ≥ 1% nuclear staining. HER2 status was scored on a scale of 0-3+ in accordance with the American Society of Clinical Oncology/College of American Pathologists guidelines. The Ki-67 proliferation index was determined by calculating the percentage of positively stained nuclei.

Based on these results, tumors were categorized into molecular subtypes as follows: Luminal A (ER-positive and/or PR-positive, HER2-negative, and Ki-67 < 14%), Luminal B (ER-positive and/or PR-positive, HER2-negative or HER2-positive with Ki-67 ≥ 14%), HER2-enriched (ER-negative, PR-negative, and HER2-positive), and triple-negative breast cancer (TNBC); ER-negative, PR-negative, and HER2-negative).

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA). Data normality was assessed using the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation or median (range), and categorical variables as frequencies and percentages. The Mann-Whitney U test was applied to compare ADC values between benign and malignant lesions. The Wilcoxon test was applied to evaluate the differences in mean ADC values among molecular subtypes. The Bonferroni test was used as a post-hoc analysis. Spearman correlation coefficients were calculated to assess the relationships between ADC values and IHC biomarkers (ER, PR, HER2, Ki-67). Differences in ADC values between metastatic and benign lymph nodes were analyzed using the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analysis was performed to determine optimal ADC cut-off values for differentiating malignancy, and the area under the curve, sensitivity, and specificity were calculated. A two-tailed *P* value < 0.05 was considered statistically significant.

RESULTS

A total of 159 breast lesions from 148 female patients were analyzed. The median age of our cohort was 48 years (range: 25-75 years). The median age of patients with malignant tumors was 52 years (range: 38-75), whereas that of patients with benign lesions was 42 years (range: 25-62). The median age of patients with malignant tumors was substantially greater than that of individuals with benign lesions ($P = 0.035$).

Among them, 56 lesions (35.2%) were malignant and 103 lesions (64.8%) were benign. Eight patients with multiple lesions underwent needle biopsies, all of which showed benign cytology. The mean tumor diameter of all the 159 breast masses assessed in the study was 28 ± 8.3 mm (range 5-70 mm). All 56 malignant breast tumors exhibited hyperintense signal features on DWI.

The histopathological distribution of malignant lesions included 6 cases of ductal carcinoma in situ (10.7%), 41 cases of invasive ductal carcinoma (73.2%), and 9 cases of invasive lobular carcinoma (16.1%).

When comparing the median ADC values of malignant lesions (0.92×10^{-3} mm^2/s) to those of benign lesions (1.66×10^{-3} mm^2/s), it was found that the malignant ones had significantly lower values ($P < 0.001$, Figure 2). ROC curve analysis indicated that the optimal ADC cut-off value for differentiating malignant from benign masses was 1.24×10^{-3} mm^2/s (sensitivity 95.2%, specificity 89.1%). Figure 3 illustrates a specific case of a malignant tumor.

Upon classifying malignant tumors into molecular subtypes according to IHC markers, we detected 6 (10.7%) luminal A tumors, 38 (67.9%) luminal B tumors, 4 (7.1%) HER2-enriched tumors, and 8 (14.3%) triple-negative tumors.

Hormone receptor-positive tumors exhibited a statistically significant reduction in median ADC value compared to hormone receptor-negative tumors (0.90×10^{-3} mm^2/s versus

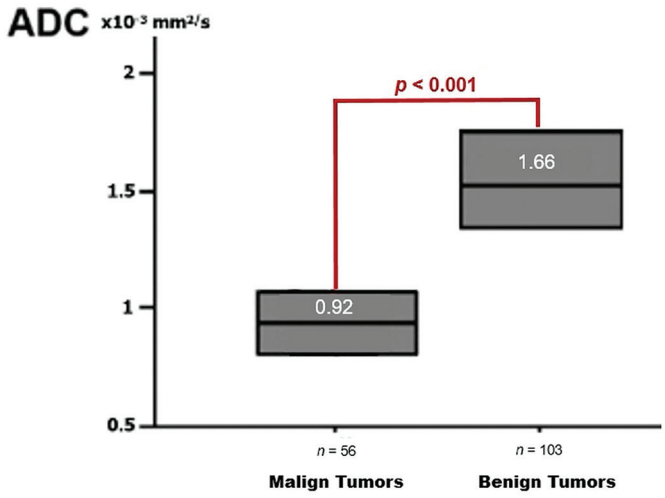

Figure 2. Median ADC values for benign and malignant breast tumors.

ADC, apparent diffusion coefficient

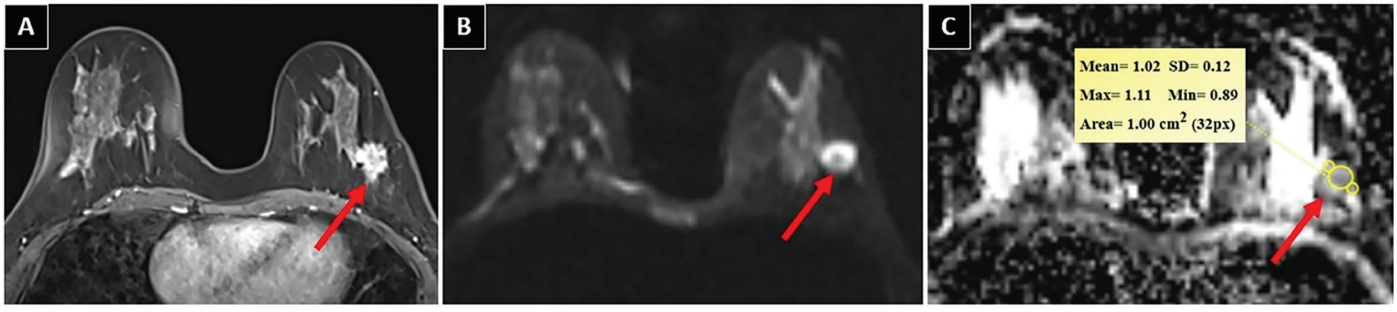

Figure 3. Invasive ductal carcinoma in a 48-year-old female patient. A) An irregularly contoured mass with significant contrast enhancement is noted in the upper outer quadrant of the left breast on post-contrast breast MR image (red arrow). B) The lesion exhibits hyperintensity on diffusion-weighted imaging (red arrow). C) The ADC value obtained from ROI measurement of the lesion on ADC maps is 1.02 ×10-3.mm²/s.

ADC, apparent diffusion coefficient; MR, magnetic resonance; ROI, region of interest, SD, standard deviation.

1.08×10^{-3} mm²/s, P = 0.03). No significant association was found between HER2 status and median ADC values. Furthermore, in the independent assessment, lower median ADC values were observed in patients with a high Ki-67 index (P = 0.015). The triple-negative breast cancer (TNBC) subtype had the highest median ADC value (1.13 × 10^{-3} mm²/s) compared with other molecular tumor subtypes.

An evaluation of the axillary lymph nodes was conducted in 26 patients with malignant tumors. Median ADC values of ipsilateral pathologically proven metastatic nodes were significantly lower than those in contralateral benign lymph nodes (0.78 versus 1.82×10^{-3} mm²/s; P < 0.001, Figure 4). Figure 5 shows a metastatic axillary lymph node.

DISCUSSION

In this retrospective study, we demonstrated that diffusion-weighted MRI and quantitative ADC measurements can effectively distinguish between benign and malignant breast lesions, while also providing valuable information about tumour biology and axillary lymph node status.

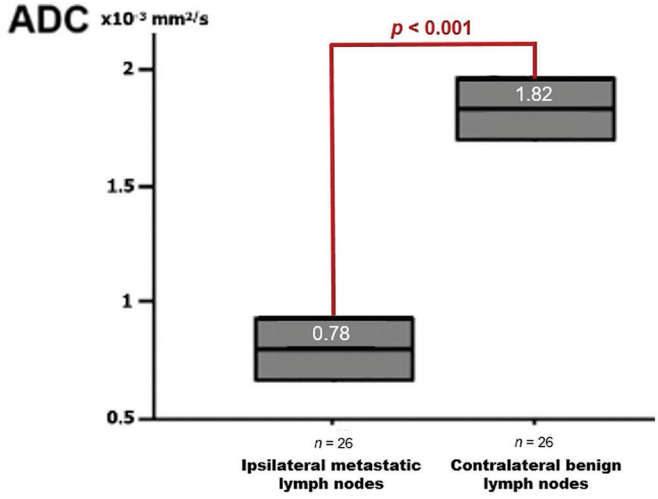

Figure 4. Median ADC values for benign and malignant axillary lymph nodes.

ADC, apparent diffusion coefficient

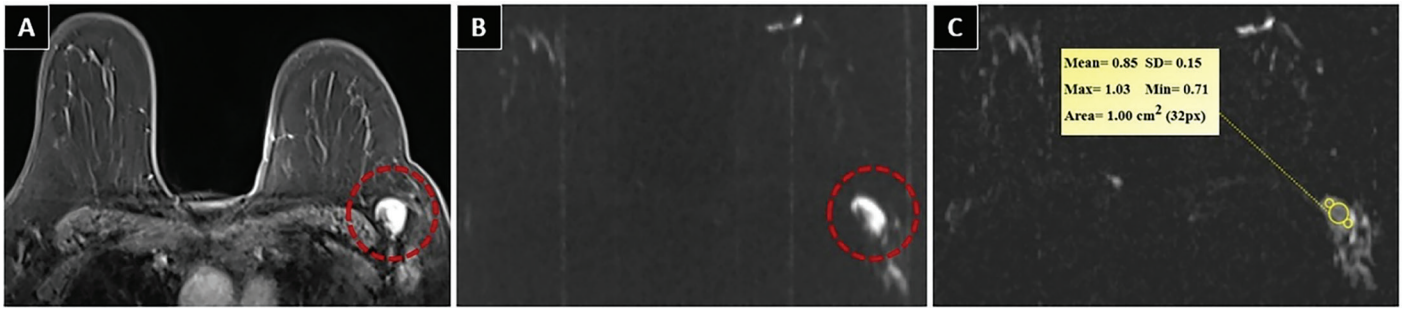

Figure 5. Metastatic left axillary lymphadenopathy in a 51-year-old female patient. A) The post-contrast breast MR image shows lymphadenopathy with a thickened and markedly enhanced cortex in the left axilla (red circle). B) The lesion exhibits hyperintensity on diffusion-weighted imaging (red circle). C) The ADC value obtained from ROI measurement of the cortex on ADC maps is 0.85 ×10−3 mm^2/s.

ADC, apparent diffusion coefficient; MR, magnetic resonance; ROI, region of interest, SD, standard deviation.

This study revealed that malignant breast tumors demonstrated markedly lower ADC values than benign lesions. The optimal ADC threshold value of 1.24 × 10^{-3} mm^2/s demonstrated excellent diagnostic performance, with 95.2% sensitivity and 89.1% specificity, aligning with other meta-analyses that indicate the high accuracy of DWI in breast tumor characterization.[6,10] This observation supports the established scientific rationale that increased cell density and reduced extracellular space restrict the movement of water molecules, thereby reducing ADC values.[10] In an early study on breast cancer diagnosis, Marini et al.[11] found a sensitivity of 80% and a specificity of 81% at a cut-off ADC value of 1.1 × 10^{-3} mm^2/s. In an alternative investigation, the ADC threshold value most similar to that in our study was determined for differentiating malignant from benign breast tumors, resulting in a proposed cut-off value of 1.23 × 10^{-3} mm^2/s.[12]

The main reason for selecting b values of 0 and 1000 in DWI is to increase the comparability of our results with those reported in the literature we cite on this subject.[2,5]

Beyond lesion characterisation, this study showed that ADC values can predict pathological biomarkers indicative of molecular features in malignant tumors. Hormone receptor-positive tumors showed markedly lower ADC values compared with hormone receptor-negative lesions. This phenomenon can be attributed to the comparatively elevated histopathological grade and increased cellularity commonly observed in luminal B type tumors. The results suggest that quantitative ADC measurements of lesions in patients with breast cancer can serve as a non-invasive method for grading and prognostic assessment.[8,13]

Moreover, our investigation found that malignancies with an elevated Ki-67 proliferation index had reduced ADC values, consistent with the biological mechanism whereby aggressive tumours with rapid cellular turnover exhibit increased diffusion restriction.[14,15] Conversely, no significant association was detected between HER2 status and ADC values. These data align with prior reports suggesting that HER2 status is not a reliable predictor of diffusion.[8,16]

Interestingly, the highest median ADC levels were observed among TNBC subtypes. While the literature indicates that TNBCs demonstrate an aggressive clinical course, it is highlighted that diffusion restriction diminishes due to the necrotic or heterogeneous microarchitecture of the tumors, leading to elevated ADC values. Aggressive tumors are expected to exhibit low ADC levels; nevertheless, the paradox seen in TNBCs is believed to stem from microarchitectural attributes. Areas of tumor necrosis signify a reduction in tumor cellularity, accompanied by enhanced diffusion, signal attenuation, and elevated ADC values on DWI.[8,17]

Another noteworthy finding concerns the evaluation of axillary lymph nodes and the proven benefit of DWI in axillary staging for breast cancer patients. The ADC values of pathologically proven metastatic lymph nodes were markedly lower than those of contralateral benign lymph nodes. This underscores the potential value of DWI in both the evaluation of the primary tumor and nodal staging. An increasing body of studies underscores that DWI is a non-invasive technique for evaluating lymph nodes, especially when the use of contrast agents is contraindicated or to guide targeted biopsies. Nevertheless, as highlighted in the literature, repeatability challenges in ADC measurements persist. Consequently, further comprehensive prospective research will be necessary to confirm the reliability of DWI in nodal staging.[9,18]

The strengths of the study include the use of a standardized imaging protocol, histological verification of all lesions, blinded evaluations by two observers with disagreements resolved by consensus, and the establishment of carefully defined, rigorous inclusion criteria. Moreover, a comprehensive evaluation of biomarker correlations and nodal analyses contributes to the existing literature. Nonetheless, there are numerous limitations. The retrospective design, modest single-center sample size, and manual ROI placement may limit the generalizability of our results. Additionally, only specific b-values were assessed, and advanced diffusion models, including intravoxel incoherent motion and diffusion kurtosis imaging, were not included. The lack of evaluation of inter-observer agreement is a limitation of our study. Another important limitation is the unequal numbers of patients across the IHC subgroups. Although most of our results are statistically significant, this situation may reduce statistical power. Ultimately, bias in lesion selection may arise from the exclusion of small lesions that are not detectable on DWI.

In clinical practice, we emphasize the significance of DWI as a biomarker for non-contrast imaging. The correlation between ADC and tumor aggressiveness supports the potential use of DWI in personalized oncology. Further investigations ought to encompass larger patient cohorts, integrate advanced diffusion models, and examine radiomics-based methodologies to enhance prognostic efficacy. Studies increasingly indicate that artificial intelligence technologies provide substantial for image construction and analysis in MRI.[19] Therefore, artificial intelligence technologies should also be included in future studies.

The study found that DWI and ADC enhance the evaluation of breast cancer. Breast malignancy, hormone receptor positivity, and proliferative activity are associated with lower ADC values. Additionally, ADC values may indicate nodal metastases. Notwithstanding its limitations, our findings suggest that DWI could be used in clinical practice as a safe, reliable biomarker if supported by large, multicenter trials.

Ethics

Ethics Committee Approval: The study received ethical approval from the Clinical Research Ethics Committee at Erzincan Binali Yıldırım University (protocol number: EBYU-KAEK-2024-17-008-412224, date: 05.12. 2024).

Informed Consent: Retrospective study. All patients have provided written informed consent for their data to be included in the MRI studies database and used in scientific research.

Footnotes

Author Contributions

Concept and Design – K.B.M., A.S.Ç.; Data Collection and/or Processing – A.S.Ç., M.F.Ö.; Analysis and/or Interpretation – K.B.M.; Literature Review – A.S.Ç.; Writing, Reviewing and Editing – K.B.M., A.S.Ç.

Conflict of Interest: The authors declare no conflict of interest.

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