

## Role of DWI in speculating molecular subtype and nuclear grade of malignant breast lesions.

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### Abstract

**Background:** Diffusion weighted imaging (DWI) is an adjunct sequence to DCE-MRI of breast imaging in differentiating benign and malignant findings. Many previous studies have established that malignant lesion shave low ADC values than benign lesions. DWI is sensitive to microstructural tissue properties including cell density, cellular organization, and cell membrane integrity.

ADC mapping helps in identifying pathological lesion sin the breast. Due to increased cellularity and more restricted diffusion, malignant breast lesions commonly exhibit hyperintense signal on DWI with low ADC values relative to normal breast parenchyma. These ADC value can be taken in to account inspeculating molecular subtype and nuclear grade in breast malignancy.

**Materials and Methods:** The present study is a cross-sectional, observational study undertaken to assess the "Role of diffusion weighted imaging (DWI) in the evaluation of breast malignancy," with patients being referred to the department of radiology at NRI Medical College and GH Chinakakani.

A 1.5 Tesla MRI was used to examine all of the study participants.

**Results:** In our study, out of 50 patients, 62% were males and 38% were females. Infiltration of tracts was seen in 38% of the 50 cases, displacement in 38% of the cases, disruption in 24% of the cases, and edema in 14% of the cases. There was no significant decrease in FA values as per T tests.

**Conclusions:** Diffusion weighted imaging (DWI) is helpful in predicting molecular subtype and nuclear grade of breast cancer.

**Keywords:** DWI, apparent diffusion coefficient (ADC), nuclear grade, molecular subtypes, breast cancer (BC), luminal type A, luminal type B, Her2 enriched, Basal like, triple negative, dynamic contrast enhanced magnetic resonance imaging(DCE-MRI)

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### I. Introduction

Breast cancer is commonest cancer among women globally. It is aheterogeneous medical condition with various molecular subtypes havingvarying response to therapy and prognosis. Conventional criteria for treatmentchoices include size of tumor, grade in histology, lymph node involvement,and metastasis. However, subjects with same stage of cancer and identicalhistopathological features can show differing clinical features and prognosis.

Novel technologies in expression analysis with DNA microarray providedinformation on molecular subtypes named Luminal A, Luminal B, Humanepidermal growth factor receptor 2 (HER2) positive and triple-negative<sup>1-2</sup>.

- Luminal A will show a low proliferation index (Ki-67), that constitutes for 50-60% of all breast cancers. It shows best prognosis.
- Luminal B shows high expression of Ki-67 index, and constitutes for 20 % of BC cases. It has a poor prognosis compared to Luminal A. Luminal B do not over-express HER2.
- HER2+ types account for 10% of BCs and are featured by the low expression of hormone receptors. They show more expression of HER2/neu gene.

○ Triple-negative subtype constitutes for 7-16% of breast cancers cases and shows lack of expression of hormone receptors and low expression of HER2+. It shows more expression of cytokeratin genes of high molecular weight. It was usually less differentiated invasive carcinoma and constitutes for 70% of BCs.

○ HER2+ and Triple-negative show better responses to chemotherapy but have worst survival rates.<sup>3-5</sup> Dynamic contrast-enhanced magnetic resonance imaging (DCEMRI) is an excellent imaging technique for evaluating BC patients before planning for surgery to know the extent of disease. DCEMRI with gadolinium-based contrasts is now the most sensitive identification technique for diagnosis and it acts as a potential imaging adjunct to mammography and ultrasound (USG). It helps in detecting lesions that are not visualized by other methods.

Diffusion weighted imaging (DWI) is an adjunct sequence to DCE-MRI of breast imaging in differentiating benign and malignant findings. Many previous studies have established that malignant lesions have low ADC values than benign lesions. DWI is sensitive to microstructural tissue properties including cell density, cellular organization, and cell membrane integrity.

ADC mapping helps in identifying pathological lesions in the breast. Due to increased cellularity and more restricted diffusion, malignant breast lesions commonly exhibit hyperintense signal on DWI with low ADC values relative to normal breast parenchyma. These ADC values can be taken into account in speculating molecular subtype and nuclear grade in breast malignancy.

BC now crossed lung cancer as leading global cancer in 2020, with around 2.3 million new cases, constituting 11.7% of all cancer cases<sup>6</sup>. Global burden may cross 2 million by the year 2030<sup>7</sup>. The incidence rose significantly, around 50% from 1965 to 1985<sup>8</sup>. The incidence of BC increased by 39.1% from 1990 to 2016 in India, with the increase observed in every state of the country<sup>9</sup>. Globocan data of 2020 states that, BC accounts for 13.5% of all cancers and 10.6% of all deaths<sup>10</sup>.

The current study was done at our hospital named NRI Medical College and General Hospital, Chinakakani, which is equipped with 1100 beds. All necessary facilities were available in our department for checking all the study parameters. Around 80 women presented to the Radiodiagnosis department with suspected breast carcinoma during the study duration. From these patients, study patients were recruited.

## **II. Materials and Methods**

This cross-sectional, observational study was carried out on patients referred to Department of Radio-diagnosis at NRI medical college and general hospital, Chinakakani, Guntur, Andhra Pradesh from April 2021 to April 2022. A total 50 adult female subjects of aged  $\geq 18$ , years were for in this study.

**Study Design:** Cross-sectional, observational study.

**Study Location:** Radio-diagnosis at NRI medical college and general hospital, Chinakakani, Guntur, Andhra Pradesh.

**Study Duration:** 12 months from April 2021 – April 2022.

**Sample size:** 50 patients.

**Sample size calculation:** As per the American cancer society, the prevalence of breast cancer among women is 30%. The sample size was calculated as:  $N = Z^2 PQ/E$ . (N-Sample size; P-Prevalence=30%; Q=1-P; E-Error: 10%)

We assumed 85% confidence limits. This gave an N value of 44. Hence, 44 is taken as the minimum size. We included 50 subjects in the current study, considering few losses for follow up.

**Subjects & sample selection method:** The study population included female subjects with complaints in breast referred to department of Radio-diagnosis from departments of general surgery and oncology at NRI medical college and general hospital from April 2021 to April 2022.

### **Inclusion criteria:**

1. Patients with morphological features of BI-RADS 4b, 4c, 5 on sonomammography or x-ray mammography.
2. Patients with biopsy proven malignancy - BI-RADS 6.
3. Patients aged above 18 years.
4. Patients who provided informed consent to participate in the study.

### **Exclusion criteria:**

1. Pregnant and lactating women.
2. Patients with pacemakers, prosthetic heart valves, cochlear implants or any metallic implants.
3. Patients having history of claustrophobia.
4. Patients with prior surgery in the breast, hormonal therapy, radiation or chemotherapy.
5. Patients with previous anaphylactoid reaction to a gadolinium-based contrast.
6. Patients with altered renal function test and estimated glomerular filtration rate  $< 30$  ml/min/1.73 m<sup>2</sup>.

7. Patients with severe renal or hepatic impairment.

Exclusion criteria were based on oral history from patients, available medical records.

**Procedure methodology**

- Eligible patients were included after getting approval from the institutional ethics committee.
- Assurance was provided regarding the maintenance of confidentiality.
- All patients were investigated on 1.5 Tesla SIGNA EXPLORER MRI machine (General Electrical medical systems,NRI general hospital) with dedicated bilateral breast 16-channel coil and no compression.
- For MRI examinations of breast, patient was positioned in prone position for image acquisition.
- Imaging parameters: Field of view (FOV) 300-360 mm and slice thickness 2-5mm were used.
- The following sequences were obtained: axial - T1, T2, T2 STIR, DWI. Sagittal – STIR, both breasts.
- Diffusion weighted images were obtained using diffusion weighted echo-planer imaging sensitizing diffusion gradients with b value of 800s/mm<sup>2</sup>.
- Dynamic study Post gadolinium T1W Fat sat (Vibrant Multiphase) were obtained in axial plane. Pre-contrast fat-suppressed T1W gradient echo images were first obtained and this was followed by intravenous contrast injection.
- Contrast material varied over time. MultiHance (GdDTPA-BMA) 0.2mmol/kg body weight was injected as a bolus, followed by a flush of 20 ml of saline.
- Gradient-echo images were obtained at 1-minute intervals, till 6 minutes.
- Post processing was done by digitally subtracting the precontrast images from the sequential post contrast images.
- Kinetic analysis was done using the mean curve technique.
- MRI interpretation was done analyzing the pre-contrast, post contrast images and post processing data.
- After identifying the lesion on post contrast images, multiple ROIs were drawn on ADC map and the lowest ADC values were taken into consideration and correlated with histopathology, nuclear grade and immunohistochemistry.

**Statistical analysis**

The data collected was entered in Excel 2019 and analysis was carried out using excel 2019 and software called Epi info version 7.2.5. The results were expressed in the form of descriptive and inferential statistics.

Probability value below 0.05 was considered statistically significant. Frequencies, percentages were also used. Continuous variables were calculated by using mean and SD. Categorical parameters were determined using chi-square test.

**III. Results**

**Age:**The mean age of patients was 55.6years. Age of patients ranged from 35years to 76 years.

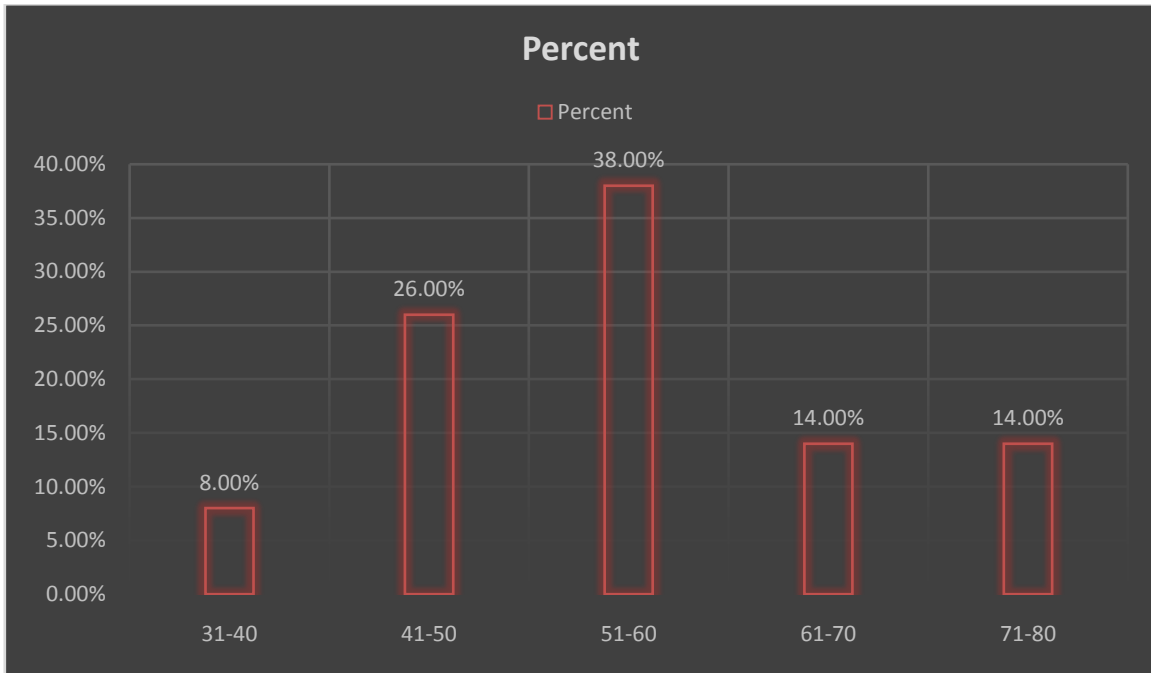
Obs	Total	Mean	Std Dev
50.0000	2780.0000	55.6000	10.4530
Minimum	Median	Maximum	Mode
35.0000	54.5000	76.0000	50.0000

**Table 1: Mean age of patients**

**Age group:**38% of patients were aged 51-60 years, 26% were aged 41-50 years,14% were aged 61-70 years, and 14% were aged 71-80 years.

Age group	Frequency	Percent	Cum. Percent
31-40	4	8.00%	8.00%
41-50	13	26.00%	34.00%
51-60	19	38.00%	72.00%
61-70	7	14.00%	86.00%
71-80	7	14.00%	100.00%
<b>Total</b>	50	100.00%	100.00%

**Table 2: Age group**

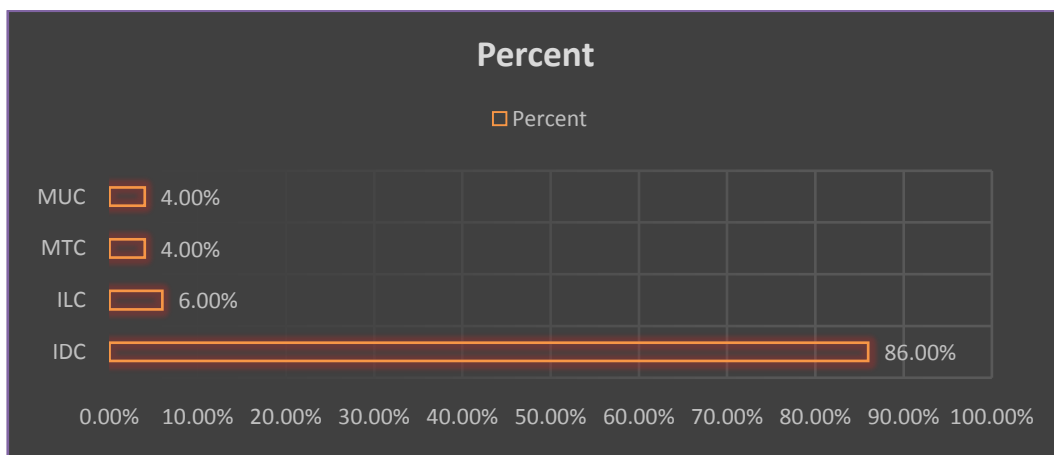


**Graph 1: Age group of patients**

**DWI:**The mean DWI was 0.6.It ranged from 0.2 to 1.2.

Obs	Total	Mean	Std Dev		
50.0000	31.5600	0.6312	0.269		
Minimum	25%	Median	75%	Maximum	Mode
0.2000	0.4000	0.6000	0.8000	1.2000	0.4000

**Histology:**86% patients had invasive ductal Carcinoma (IDC).IDC was the most common diagnosis at HPE. Invasive lobular carcinoma(ILC) was seen in 6% of patients. Mucinous carcinoma was seen in 4% of patients and metaplastic carcinoma in-situ in 4% of patients.



**Graph 2: Histology of mass**

**Nuclear grade:**It is given according to Modified Scarff-Bloom-Richardson histological grading. 4% of patients had Grade 1 lesions,52% patients were having nuclear Grade 2 mass, 44% patients were with nuclear grade 3 mass.

Modified Scarff-Bloom-Richardson histological grading

Grade 1: (Score 3-5) is low grade number which usually means the cancer is slower-growing and less likely to spread.

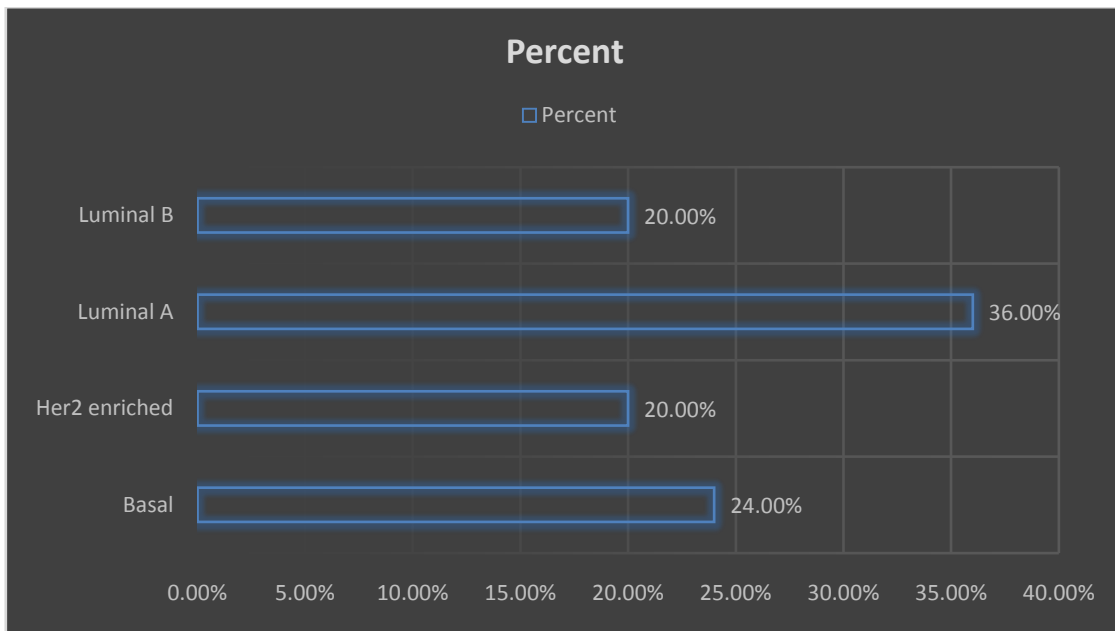
Grade 2: (Score 6-7) is taken as an intermediate stage, which indicates that cells grow rapidly than grade 1.

Grade 3: (Score 8-9) It is a high grade number with faster-growing cancer that's more likely to spread.



**Graph 3: Nuclear grade of mass**

**Molecular subtype:** 36% of patients were seen having luminal A molecular subtype, 24% were having basal subtype, 20% were having her-2 enriched subtype, 20% were having luminal B molecular subtype in the current study among 50 patients included.



**Graph 4: Molecular subtype**

**Correlation between DWI and nuclear grade:** Most of the tumours in our study showed Grade 2 or Grade 3 nuclear grade at histopathological examination. Grade 3 tumours commonly had ADC values in the range of  $0.2-0.4 \times 10^{-3} \text{ mm}^2/\text{s}$  whereas Grade 2 tumours had ADC values in the range of  $0.5-0.7 \times 10^{-3} \text{ mm}^2/\text{s}$ .

**Association between DWI and molecular subtypes:** There was significant correlation between DWI and molecular subtypes as per ANOVA analysis. Luminal type B carcinomas with her2 receptors had low ADC values followed by luminal type B with only oestrogen and progesterone receptors and luminal type A carcinomas. This ranged in between  $0.2-0.5 \times 10^{-3} \text{ mm}^2/\text{s}$ . Her2 enriched and basal like carcinomas had ADC values in the range of  $0.5-1.2 \times 10^{-3} \text{ mm}^2/\text{s}$ .

#### IV. Discussion

This study was a cross-sectional, observational study conducted at NRI Medical College & General Hospital, Chinakakani- a tertiary care center with well-equipped facilities. The study was conducted for 12 months from April 2021 – April 2022.

Patients aged above 18 years with suspected breast masses, who signed informed consent form were included.

Patients with incomplete data, history of claustrophobia, cardiac pacemakers, prosthetic heart valves, cochlear implants or any metallic implants, pregnant and lactating were excluded. 50 patients were included as per the sample size.

Age, MRI diagnosis, DWI, kinetic curve assessment, HPE findings, molecular subtype, ER, PR, Her 2 status, Ki-67 index, were assessed for all patients. Numerical parameters were assessed using Mean and SD.

Statistical tests were done using Epi info software, Microsoft Excel 2019.

Most of the parameters were expressed as frequencies and percentages. Age was given as Mean and SD.

Association between DWI with molecular subtype was done using ANOVA test.

##### **Molecular subtypes:**

Luminal-type (which is ER or PR-positive) breast cancer is the most common type of BC. It is classified as luminal-A and luminal-B subtypes. It was defined by low- (Ki-67 below 14) and high- (HER2-positive or Ki-67 more than 14) proliferation subtypes.<sup>11-13</sup>

Hormone receptor negative BCs include HER2-positive and basal subtypes. They were usually with more Ki-67 indexes.<sup>14-18</sup>

HER2 is over expressed in around 20% of BC cases and is linked to a poor prognosis, but it shows good response to HER2 therapies<sup>19</sup>.

Basal-like BCs are Triple negative BCs on semi-quantitative scoring<sup>20</sup>. BC subtype can be easily diagnosed by immunochemical staining of biopsy sample, but receptor expression may modify during treatment.

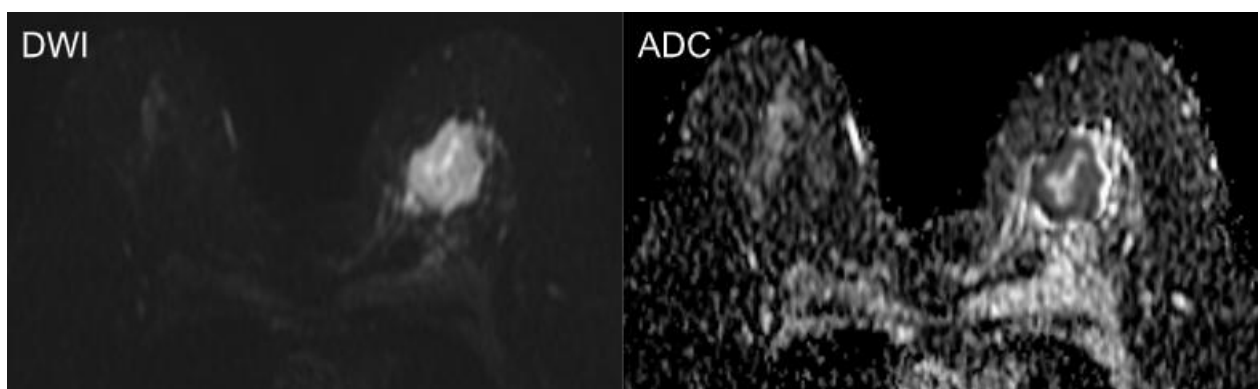
Reiko et al. demonstrated the potential for DWI to be used in the assessment of cancer extension.

Jin et al. stated that the use of DWI offers increased specificity with b values ranging from 600 to 1000 s/mm<sup>2</sup>.

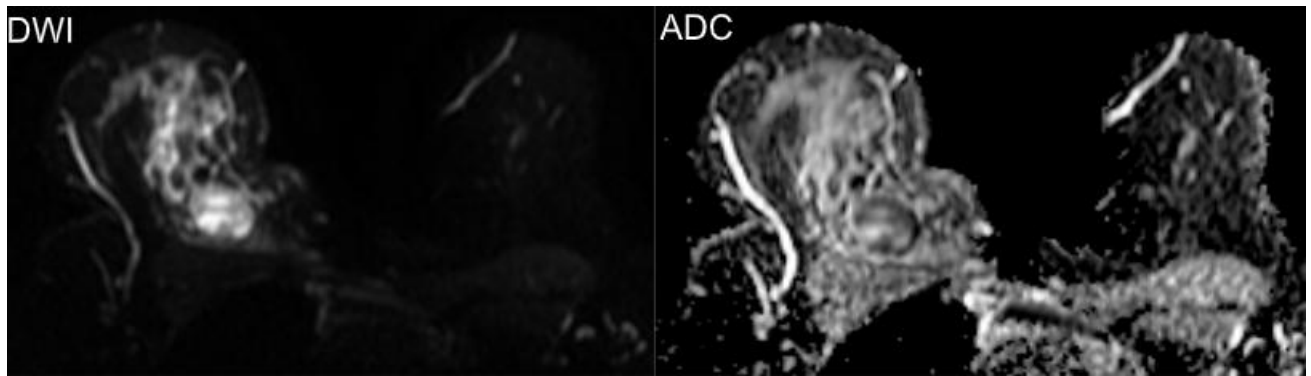
W. Abdulghaffar et al. concluded that DWI is easy to obtain in short scan time and easy to evaluate, and ADC values can differentiate between benign and malignant breast lesions with high sensitivity and specificity. DWI is a problem-solving sequence in patients with contraindication to contrast media.

Lima et al. stated that DWI has shown to add value in tumour detection, characterization, and prediction of treatment response.

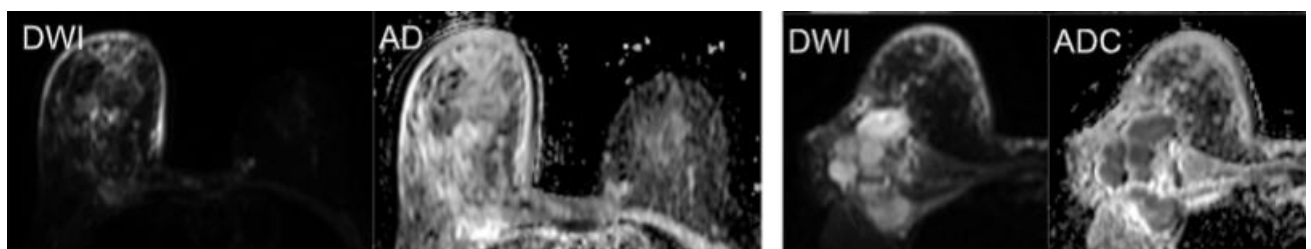
DWI provides details on prediction of nuclear grade and molecular subtypes of breast carcinoma.



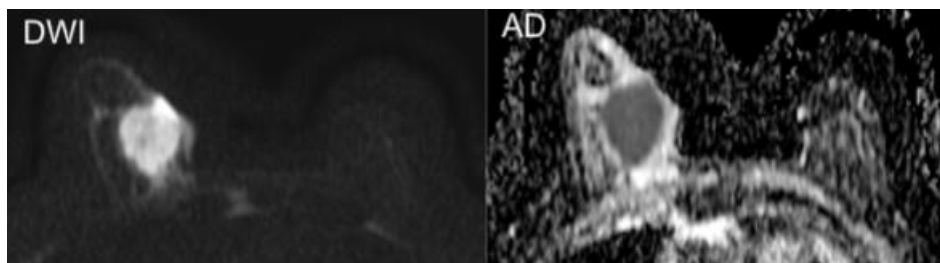
**Image 1:** Invasive carcinoma – non special type of Luminal type A molecular subtype with nuclear Grade 2. An irregular mass with non-circumscribed margins at 9-12’o clock position showing diffusion restriction with low ADC values in the range of  $0.5-0.8 \times 10^{-3} \text{ mm}^2/\text{s}$ .



**Image 2:** Invasive ductal carcinoma – non special type. Medullary carcinoma of Luminal type B molecular subtype with nuclear Grade 3. Round to ovoid, non-circumscribed mass in posterior third depth at 1-2'o clock position, showing diffusionrestriction with low ADC values in the range of  $0.2-0.3 \times 10^{-3} \text{ mm}^2/\text{s}$ .



**Image 3:** Invasive ductal carcinoma – non special type. Her 2 enriched molecular type carcinoma with nuclear grade 3. Right breast is diffusely enlarged in size. An irregular shapedmass with non-circumscribed margins measuring at 9-10'oclock position in middle third depth of right breast. Multiple enlarged and rounded axillary lymphnodes seen. The lesions showsdiffusion restriction with low ADC values in the range of  $0.3-0.4 \times 10^{-3} \text{ mm}^2/\text{s}$ .



**Image 4:** Unifocal metaplastic carcinoma with triple negative molecular subtype and nuclear grade 3. Exophytic, round circumscribed mass in lower inner quadrant and anteriorthird depthshowing diffusionrestriction with low ADC values in the range of  $0.3-0.4 \times 10^{-3} \text{ mm}^2/\text{s}$ .

### **V. Conclusion**

Diffusion weighted imaging and ADC maps helps in determining various molecular subtypes of breast cancer, especially in cancers expressing estrogen and progesterone receptors. Luminal A, luminal B cancers have low ADC values commonly.

Sophisticated evaluation of tumor heterogeneity, more studies on recently introduced techniques, and standardized interpretation of images can improve non-invasive breast cancer molecular subtype classification.

DWI can help to provide personalized treatment for patients with breast cancer. However, it should be an adjunct technique to DCE-MRI evaluation of breast as it can miss minute lesions. Usefulness of this technique needs further evaluation with larger studies and follow up of treatment.

The study is self-sponsored. There were no conflicts of interest.

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