Association of apparent diffusion coefficient with molecular biomarkers (ER, PR, HER2 status) in invasive breast cancer patients using diffusion weighted MR imaging

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Objectives: To determine the apparent diffusion coefficient (ADC) of breast cancer patients and its association with molecular subtypes based on expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptors (HER2).

Introduction: Breast cancer is a heterogeneous disease with considerable genotypic and phenotypic diversity (1). There are five distinct subtypes of breast cancer [luminal A and B, human epidermal growth factor receptor 2 (HER2), basal and normal like]. Luminal tumors are characterized by expression of estrogen receptor (ER), which is usually accompanied by progesterone receptor (PR) expression. Estrogen receptor is well established promoter of cell division as it causes proliferation of both normal and malignant cells. ER negative (ER-) breast cancer, accounts for 20-30% of breast cancers and has a poor prognosis (2). HER2 over-expression occurs in ~20% of breast cancers and has been associated with decreased survival which tends to be aggressive and associated with high recurrence and poor prognosis. These molecular biomarkers have become an essential component and play an important role (almost in 70% of cases) in decision-making during treatment of breast cancer patients (3). Thus, early diagnosis and understanding of the molecular features of breast tumors is important for successful treatment and to decrease the morbidity and mortality. DWI measures the apparent diffusion coefficient (ADC) of tissues, providing information on the cell density, volume of extracellular matrix and cellularity. Studies have reported that ADC have diagnostic potential in delineation of different breast tissues (malignant, benign and normal) (4,5). We hypothesize that ADC may be useful parameter to characterize and detect different breast tumor based on molecular subtypes. Therefore, in the present study, we determined the ADC values obtained from DWI in different molecular subtypes (ER, PR and HER2) of invasive breast cancer patients to get an insight into the association of ADC with the molecular heterogeneity of breast lesions.

Material and Methods: A total of 72 (45.8 ± 11.5, years) women for whom ER, PR, HER2 status available was included in the analysis. Written informed consent was obtained and Institutional ethical committee approved the study. Patients with the clinically palpable lump were subjected to FNAC for confirmation of malignancy followed by core needle biopsy. Biopsied tissue was subjected to histology and immune-histochemical examinations to determine the expression of hormonal receptors like ER, PR and HER2. Patients with HER2 expression scores 0 and 1+ were categorized as HER2-negative (HER2-) and those with the scores of 3+ were categorized as HER2/neu-positive (HER2+). 23 patients with the score of 2+ were excluded from the analysis since their data of fluorescence in situ hybridization was not available. Thus, 16 patients fall under category of HER2+ while 33 under HER2-; 34 in ER+ and 38 ER-; 36 with PR+ and 36 under PR-. DWI was carried out using a phased array breast matrix coil at 1.5 T (AVANTO, Siemens Health Care, Germany). DW images were acquired in the transverse plane using a single-shot EPI sequence with TR = 5000 ms; TE= 87 ms; FOV = 250 – 350 mm; NS = 1; EPI factor =128; acquisition matrix = 128 x 128; and slice thickness = 4 to 5 mm, without any inter slice gap. Three 'b' values of 0, 500 and 1000 s/mm^2 were used; Mean ADC were calculated using ADC map by drawing contiguous circular ROIs of five pixels (size = 0.31 cm^2) from the hypo-intense area of malignant tumor. All statistical analyses were carried out in SPSS software 16.0. Student's t-test was used to compare ADC values with the ER, PR and HER2 status.

The age and mean ADC in breast cancer patients for whom ER, PR and HER2 neu status was available (n=72)		
Groups and number of patients (n)	Age in years	ADC values (x 10^{-3} mm^2/s)
HER2/neu+ (a); n = 16	46 ± 10.8	1.08 ± 0.17
HER2/neu- (b); n = 33	45 ± 13.1	1.04 ± 0.13
ER+ (c); n = 34	49 ± 11.3*	1.02 ± 0.13
ER- (d); n = 38	43 ± 11.3*	1.03 ± 0.16
PR+ (e); n = 36	47 ± 11.6	1.04 ± 0.12
PR- (f); n = 36	44 ± 11.3	1.01 ± 0.17
* denotes p<0.05 between (c) and (d)		

Results and Discussion: Despite the improvement in the detection of breast cancer with the widespread application of various modalities, breast lesion characterization is still challenging. This study was carried out using DWI technique to evaluate the diagnostic potential of ADC in characterizing breast tumors based on various molecular subtypes. Molecular characterization of tumors is critical for identifying important genes and for improving tumor classification and diagnosis. Our retrospective analysis revealed that the ADC values in HER+, ER+ and PR+ patients were not significantly different compared to HER-, ER- and PR- patients (see Table). ER- patients had lower age as compared to ER+ patients (p=0.02). In this study we noticed low ADC values in various tumor subtypes as compared to benign and normal breast tissues reported earlier by us (4, 5). Also, there is no wide variation in ADC values among various tumors subtypes of breast cancer, thereby indicating that there is no association of ADC with HER2, ER, and PR status of patients. These observations demonstrate that in different tumor subtypes, the mean ADC is statistically insignificant. Recently, Martincich et al., (6) have reported that the median ADC values were significantly higher in ER-negative than in ER-positive tumors (1.11 vs 1.05 × 10^{-3} mm^2/s, P = 0.015). HER2-enriched tumors had the highest median ADC value (1.19 × 10^{-3} mm^2/s, range 0.950-2.090). However, our data indicated that the measured ADC was not significant but showed variation (0.7-1.4x10^{-3} mm^2/s) with the different tumor sub types which might be attributed to intratumor and intertumor heterogeneous nature of breast lesions or other molecular features of breast cancer which is influenced by genomic variation, etc,. Further, studies are required in large number of patients in each category (ER, PR and HER status) to understand the underlying mechanisms of different tumor subtypes and its association with ADC.

Refereneces: (1) Perou CM et al., *Nature* 2000; 406: 747-752; (2) Carey LA, et al., *J. Am.Med. Assoc.* 2006; 295: 2492-2502; (3) Althuis MD et al., *Cancer Epidemiolog. Biomark. Preven.* 2004; 13: 1558-1568; (4) Sharma et al., *NMR Biomed* 2009; 22: 104-13; (5) Sah et al., *Proc Intl Soc Magn Reson Med*., 20: 2012; 2984; (6) Martincich L., et al., *Eur Radiol*. 2012; 22:1519-28.