Role of choline as a biomarker of cell proliferation to differentiate HER2/neu positive and negative breast cancers patients

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Objectives: The aim of this present study was to investigate the relationship between tCho concentration and tumor volume determined prior to therapy using MRS and MRI in invasive ductal breast carcinoma patients with the Her2/neu status.

Introduction: Overexpression and amplification of HER2/neu proteins have been found to be associated with the aggressive behaviour in breast cancers. This molecular marker is important for guiding treatment choices of breast cancer patients and is over expressed in about 20% of the cases (1). HER2/neu positive lesions show poor prognosis and its overexpression is associated with the increased disease recurrence. Several in-vivo and in-vitro MR spectroscopy studies demonstrated that alterations in the levels of choline-containing metabolites like phosphocholine (PCho), glycerophosphocholine (GPC), and choline are associated with the malignant transformation of breast cancer. However, only limited literature correlating MRI and MRS characteristics of lesions with the expression of HER2/neu in breast cancer patients with contradictory results are available. Tse et al. (2) and Agrawal et al. (3) reported higher choline detection rate in HER-2/neu positive patients compared to Her-2/neu negative patients. However, a recent study reported limited role of in-vivo MRS in characterizing HER2/neu overexpression in breast cancer. An in-vitro proton MRS study documented that overexpression of HER2/neu is associated with the increased levels of tCho in a tumor cell line (4). Thus, the objective of the present study was to compare and correlate the tCho concentration and tumor volume with the Her2neu positive and negative status of breast cancer patients

Materials and methods: 53 patients with histologically proven invasive ductal carcinoma (IDC) lesions with known Her2/neu status were recruited for the study, of which 37 were HER2/neu positive, (47.2± 10.6 yrs) and 11 were HER2/neu negative, (45.7± 14.3 yrs). Written informed consent was obtained and Institutional ethical committee approved the study. Patients were evaluated clinically and tumour size was measured using Vernier callipers. MR was performed using a phased array breast matrix coil at 1.5 T (Siemens Avanto). Following the scout image, T2-weighted coronal images obtained using standard SE sequence and fat suppressed MR images in the transverse sagittal and planes. Contrast-enhanced MRI was carried out using a fat-saturated 3D FLASH where-ever indicated for appropriate localization of the lesion. The in-vivo proton MRS was carried out using a single voxel PRESS sequence prior to therapy in all these patients. Typical water peak line width ranged from 8 to 22 Hz and water+lipid suppression was achieved using MEGA pulse. The parameters used were: TR=1500 ms, TE=100 ms, averages=128, total acquisition time was 3:18 minutes. An additional spectrum of the same voxel without water and lipid suppression obtained for the concentration calculation using the water signal as internal reference. The tCho concentration was calculated using the equation reported by Baik et al for 1.5 T (5) while volume was measured using MR images using perimeter method using formula: volume=ST[A1+A2...An] (6). All statistical analyses were carried out using SPSS software 11.5.

Table-1: Comparision of tCho and tumor volume in HER2/neu (+) and HER2/neu (-) patients

	HER/neu positive (n=37)			HER/neu negative (n=11)			
	Mean ±SD	Median	Range	Mean ±SD	Median	Range	P-value
tCho(mmol/kg)	4.8±3.3	3.9	0.80-17.70	2.7±0.7	2.5	1.86-4.16	.007
Volume (cm^3)	47.9±56.1	26.7	1.07-232	45.6±48.3	25.3	3.13-149.52	0.84

Results: Figure 1 shows the box plot of tCho concentration and tumor volume in Her2/neu positive and negative breast cancer patients, while Table-1 summarizes the comparative analysis of the data obtained. The tCho concentration for HER2/neu positive cases was 4.8 ± 3.3 mmol/kg which was statistically significant higher compared to HER2/neu negative cases (2.7 ± 0.7 mmol/kg; p-value of 0.007). The tumor volume for the Her2/neu positive cases (47.9±56.1) and negative cases (45.6±48.3) were similar (p=0.84).

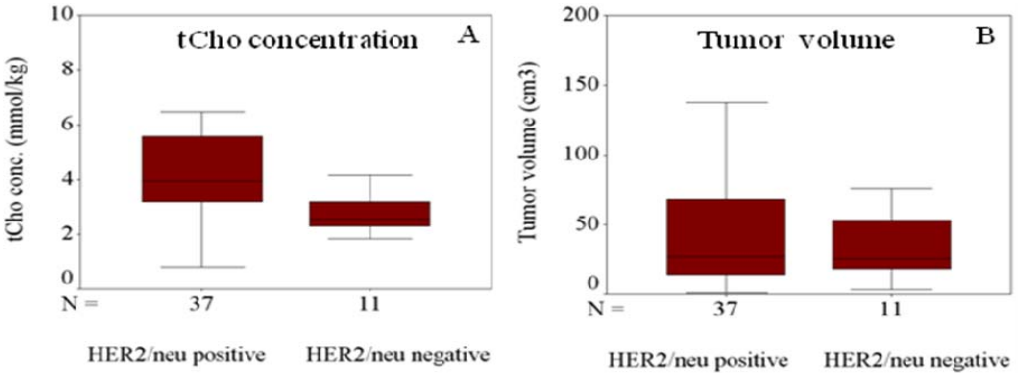

Fig-1 : A box plot showing comparison of (A) tCho and (B) tumor volume in HER2-neu-positive and negative breast cancer patients.

Discussion: HER2/neu is known for its role in the pathogenesis of breast cancer and as a target of treatment. Baek et al (7) recently reported that tCho detection rate was higher in HER2/neu positive than in the HER2/neu negative groups but statistically not significant. Our present study clearly demonstrated that tCho concentration was significantly higher in HER2/neu positive cases than in HER2/neu negative cases. Tse et al. (2) using in-vivo MRS reported that detection of Cho was related to the HER-2/neu oncogene overexpression and a false-negative spectroscopic result in their study was due to the absence of Her-2 overexpression in breast cancer. Similar results were reported by Agrawal et al. (3), however, in a small number of patients (4/7 vs. 0/8, P< 0.05). Cell line studies demonstrated a dramatic increase in the levels of cho-containing compounds with the forced overexpression of HER2/neu (4). An in-vitro study reported higher proliferation rate with the overexpression of HER2/neu in MCF7 cells (8). It was also postulated that growth factor-mediated activation of the tyrosine kinase cascade can lead to an increase in phosphocholine levels which was major components of tCho (4). It was documented that HER2 receptor mediates signalling to cancer cells and stimulates proliferation (9-11). The increase in tCho concentration observed in Her2/neu positive patients in our study might be attributed to the high proliferative activity of the Her2neu positive tumors. The tumor volume however, was not significantly different in the HER2/neu positive and HER2/neu negative groups which are in agreement with that reported by Baek at el (7) and Agrawal et al (3). The important finding of our study is that the Her2/neu overexpression is associated with the higher tCho concentration and the sensitivity of tCho detection may be related to the HER2/neu overexpression in breast cancer patients.

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