Association of total choline concentration and tumor volume in triple negative (TN), non-triple negative (nTN) and triple positive (TP) breast cancer patients: An MRI and in vivo proton MRS study

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Introduction: Triple-negative (TN) breast cancers do not express the genes for estrogen receptor (ER), progesterone receptor (PR) and Her2/neu and accounts for approximately 10% to 15% of all breast cancers. Tumors which express ER, PR and HER2/neu are defined as triple positive (TP). TN status plays an important role in guiding the treatment and also influences the prognosis of breast cancers. In-vivo MRS is a useful technique for characterization of malignant breast lesions with the observation of total choline (tCho) peak. Addition of MRS increases the specificity of MRI and studies have indicated that sensitivity of tCho detection is related to HER2/neu status of breast cancer patients (1). However, no systematic study is reported in evaluating the association of all three receptors [TN, non-triple negative (nTN) and TP status] with tCho and tumor volume. We hypothesize that this information would be useful in increasing the sensitivity and specificity of MRS and for better interpretation of MRS data. Thus, the objectives of the present study were to estimate the absolute concentration of tCho and tumor volume and to evaluate its association with TN and nTN status in infiltrating ductal carcinoma (IDC) patients.

Patients and Methods: A total of 73 (mean age = 45.5 ± 11.4; range: 25 – 70 years) women with infiltrating ductal carcinoma for whom ER, PR and HER2 status available were included in the analysis. Written informed consent was obtained from each patient and the study was approved by the Institutional ethical committee. Patients were subjected to FNAC for confirmation of malignancy followed by core needle biopsy. Histology and immunohistochemical examinations were used to determine the expression of 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+). Out of 73 patients, 8 (11%) were negative for all the three receptors forming the TN group, 65 were nTN and of which 15 patients were with TP status.MR investigations were performed using a phased array breast matrix coil at 1.5 T (Avanto, Siemens). Following the scout image, T2 coronal STIR, fat suppressed images in the axial and sagittal planes and CEMRI using a fat-saturated 3D FLASH was carried out where-ever indicated. The in-vivo proton MRS was carried out prior to therapy using a single voxel PRESS sequence with water+lipid suppression (TR=1500 ms; TE=100 ms; averages=128; TA= 3:18 min). An additional spectrum from the same voxel without water and lipid suppression was obtained for the concentration calculation using the water signal as internal reference (2). Volume was measured from MR images using formula: volume = ST[A1+A2...An]. All statistical analyses were carried out in SPSS software 16.0. Student's t-test was used to compare tCho and tumor volume with TN, nTN and TP status.

Results: tCho concentration and tumor volume for patients with TN, nTN and TP status are presented in Table 1.The retrospective analysis of the association of TN, nTN and TP status of the breast cancer patients with age, tCho concentration and volume were carried out. The tCho concentration was statistically significantly lower in TN patients (3.8 ± 1.0 mmol/kg) compared to nTN (4.7 ± 3.1 mmol/kg), (see Figures 1 and 2). Patients with TP status showed higher tCho concentration (see Table 1). However, the tumor volume was found to be insignificant between these groups (Table 1). No significant correlation of tCho concentration and the tumor volume with TN, nTN and TP status was observed.

Table 1: The tCho concentration (mmol/kg) and tumor volume (cm^3) in breast cancer patients with TN, nTN and TP status (n=73)

Groups, number of patients (n)	Age in years mean ± SD; (range)	tCho concentration mean ± SD; (range)	Tumor volume mean ± SD; (range)
TN (a) n= 8	33.4 ± 4.8 (28 - 39)#	3.8 ± 1.0* (2.4 – 5.2)	86.5 ± 80.7 (15.3 - 265.6)
nTN (b) n= 65	46.4 ± 11.1 (25 - 70) #	4.7 ± 3.1* (0.8 – 16.1)	60.6 ± 58.4 (1.1 - 268.6)
$TP (c) n = 15	46.6 ±10.30 (32-70)	5.4 ± 2.5* (2.7 - 11.0)	45.7 ± 58.9 (1.07 - 232)

* denotes p<0.05 for tCho concentration between (a) & (b). # denotes p<0.05 for age between (a) & (b).
$ denotes the sub-group of nTN group.

Discussions: Our data showed that TN group consisted of patients with younger age (28 to 39 years) with lower tCho concentration compared to the nTN patients. It is reported that TN breast cancer develops earlier in life, and consequently more often in pre-menopausal women (3,4). Chen et al. reported the association of tCho concentration with age (p=0.05) and TN status (p=0.09) (5). Higher tCho signal in TN patients compared to nTN patients has been reported (6). TN breast cancers are commonly of high nuclear mitotic grade, of larger tumor size, and they show a more aggressive expression profile with low Bcl-2 but high p53 and Ki67 expression (7,8). Recently, in aggressive basal-like xenografts, glycerophosphocholine concentrations were reported to be higher than PCho while it is reversed in luminal-like xenograft model (9). These differences were explained due to lower choline kinase expression and increased phosphatidylcholine degradation in basal-like model. Among the nTN patients, there were 15 patients with TP status and their tCho concentration was found to be significantly higher compared to TN and rest of nTN patients. There was no significant difference in the tumor volume between TN and nTN patients. The clinical significance and the relevance of TP category of patients showing higher tCho concentration as compared to TN status of patients warrants further study. Such differences suggest that the association of choline metabolism with various molecular biomarkers may be more complex and needs further investigations.

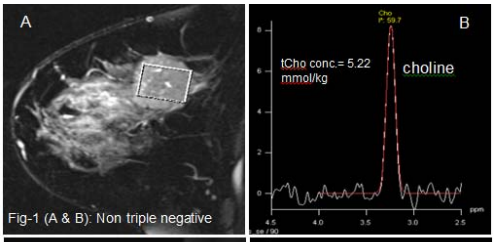

Fig-1 (A & B): Non triple negative

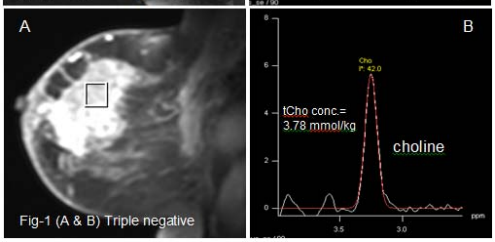

Fig-1 (A & B) Triple negative

Conclusions: Our data demonstrated the potential of quantitative proton MRS and MR imaging in characterizing malignant breast cancer based on TN and nTN status. The results presented here provide the molecular heterogeneity of breast lesions and its relation with the tumor volume and tCho concentration which may aid to better interpret the MRS data and thereby aid in clinical diagnosis.

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