Correlation of molecular biomarker (ER, PR, HER2/neu status) with total choline concentration and tumor volume in breast cancer patients: Using an MRI and in vivo Proton MRS

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Objectives: To determine the association of tCho and tumor volume in invasive breast cancer patients 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 comprising of five molecular subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2), basal like and normal like. Luminal tumors are characterized by expression of the estrogen receptor (ER), which is usually accompanied by progesterone receptor (PR) expression. Nearly 70% of breast cancers are ER+/PR+ which have a better prognosis and more treatment options than ER- cancers (1). Expression of protein kinase HER2 is up-regulated in HER2 over-expressing tumors (~20%). These molecular features of breast cancer play an important role in treatment management. Thus, early diagnosis and understanding of the molecular features of breast tumors is essential for successful treatment and in order to increase the patient survival and quality of life. In vivo magnetic resonance spectroscopy (MRS) is a unique tool that detects total choline (tCho) in malignant breast tissues and its presence has been reported to differentiate malignancy and ^{1}H MRS in a clinical setting has been reported to increase the specificity of MRI. However, variations in tCho concentration and observation of tCho peak in normal and benign lesions reduces the diagnostic value of in vivo MRS and thus understanding the reasons for these variations might help increase the diagnostic specificity. Therefore, in the present study, we determined the absolute concentration of tCho and tumor volume in different molecular subtypes (ER, PR and HER2) of invasive breast cancer patients to get an insight into the association of tCho and tumor volume with the molecular heterogeneity of breast lesions.

Material and Methods: A total of 73 (mean age = 45.5 ± 11.4; range: 25 – 70 years) women for whom ER, PR, HER2 status available were included in the analysis. Written informed consent was obtained from each patient and controls and the study was approved by the Institutional ethical committee. 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+). 26 patients with the score of 2+ were excluded from the analysis since their data of fluorescence in situ hybridization was not available. Thus, 13 patients fall under the category of the HER2+ while 34 under HER2-, 35 in ER+ and 38 ER-, 37 with PR+ and 36 under PR-. Following the scout image, T2-weighted STIR coronal, fat suppressed images in the axial, sagittal planes and CE-MRI using 3D FLASH were 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 with TR=1500 ms, TE=100 ms, averages=128, TA= 3:18 minutes. 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. tCho concentration was calculated using the equation reported by Baik et al for 1.5 T (2), while volume was measured 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 the ER, PR and HER2 status.

The concentration of tCho (mmol/kg) and tumor volume (cm^3) in breast cancer patients for whom ER, PR and HER2 neu status was available (n=73).

Groups and no. of patients (n)	tCho concentration (mean ± SD) range	Tumor volume (mean ± SD) range
HER2/neu+ (a) n = 13	3.8 ± 1.2(1.7 – 6.3)	94.4 ± 71.9(20.5 – 232.0)
HER2/neu- (b) n = 34	4.4 ± 2.9(1.0 – 11.8)	83.6 ± 70.2 (8.7 – 268.6)
ER+ (c) n = 35	5.0 ± 2.7(0.8 - 11.8)	49.5 ± 48.4$^{\$}$(1.1 – 232.0)
ER- (d) n = 38	4.3 ± 2.8(1.0 - 16.1)	93.6 ± 83.1$^{\$}$(2.8 – 385.8)
PR+ (e) n = 37	5.0 ± 2.8(1 – 11.8)	71.4 ± 69.3(1.07 – 268.6)
PR- (f) n = 36	4.7 ± 2.9(1.0 - 16.1)	66.7 ± 62.6(1.8 - 265.6)
$ denotes p<0.05 for tumor volume between (c) and (d).		

Results: A retrospective analysis of tCho concentration and the tumor volume was carried out according to the HER2, ER and PR status of the patients presented in Table. Figure 1 represents the box plot showing the variation of tCho concentration and and tumor volume with ER and HER2/neu status. The concentration of tCho in HER2+ (3.8 ± 1.2), ER+ (5.0 ± 2.7) and PR+ (5.0 ± 2.8) patients was not significantly different as compared to HER2 – (4.4 ± 2.9), ER-(4.3 ± 2.8) and PR- (4.7 ± 2.9) patients. The tumor volume in ER+ patients was statistically significantly lower compared to patients with ER-. Also no statistically significant difference in the tCho concentration and the tumor volume was observed with the PR status of patients (Table).

Discussions: In the current study concentration of total tCho levels measured showed a wide variation with the different sub types of tumor but no significant difference was observed among various molecular subtypes. The wide range of tCho concentration observed might be attributed to the heterogeneous nature of the breast lesions or other molecular features of breast cancer. Further tumor volume was found to be significantly larger in ER- group than in ER+ group. Chen et al. reported larger tumor volumes with markedly higher micro-vessel density in ER- cancers with no difference in Cho levels (3). Koukourakis et al. reported an inverse association of micro-vascular density with ER expression (4). The higher proliferative activity associated with ER- cancers may be one reason for larger tumor volumes observed in our patients (5). Our data demonstrated the potential of quantitative ^{1}H MRS and MR imaging in characterizing malignant based on different sub-types.

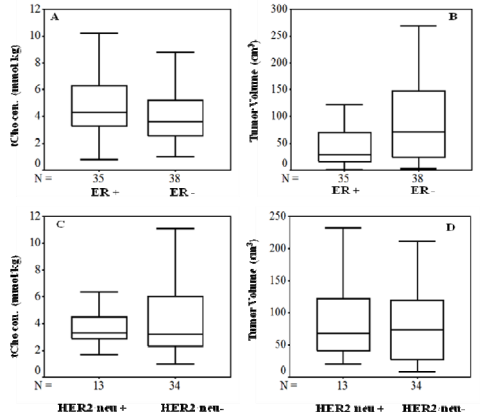

Figure 1: Box plot showing the (A) variation of tCho concentration in ER positive and negative breast cancer patients (p = 0.27); (B) tumor volume in ER positive and negative breast cancer patients (p = 0.38); (C) tCho concentration in HER2 positive and negative patients (p = 0.16) and (D) tumor volume with HER2 positive and negative breast cancer patients (p = 0.32).

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