Cut-off values of the absolute concentration of total choline for the differentiation of early breast cancer, locally advanced breast cancer, benign and normal breast tissues using proton in vivo MRS in large cohort of women

Rani G Sah[1], Uma Sharma[1], Rajinder Parshad[2], Vurthaluru Seenu[2], and Naranamangalam Raghunathan Jagannathan[1]

[1]*Department of NMR & MRI Facility, All India Institute of Medical Sciences, New Delhi, Delhi, India,* [2]*Department of Surgery, All India Institute of Medical Sciences, New Delhi, Delhi, India*

Introduction: Early detection and differentiation of malignant and benign lesions are vital in the diagnosis of breast cancer for appropriate treatment as it improves the quality of life and the overall survival of patients. Studies have demonstrated that alterations in the levels of choline-containing metabolites (tCho) are found to be associated with malignant transformation in breast cancer. However tCho was detected in benign, normal breast tissues and in lactating breast. This emphasizes the need for quantifying the concentration of tCho for the differentiation of various breast tissues. The aim of the present study was (a) to determine a cut-off value of tCho concentration for the differentiation of malignant (early and locally advanced breast cancer, LABC), benign and normal breast tissues using ROC analysis in a large cohort of women, and (b) to compare the tCho concentration with the tumor stages.

Material and Methods: 234 women including 167 cytologically proven infiltrating ductal carcinoma (IDC) patients [early breast cancer (EABC; stage IIA), n = 37 (47.6±11.6 yrs) and LABC patients with stages IIB and III (A, B, & C); (n = 130 with mean age (45. 6±10.4)]; 40 benign (31.0 ± 6.2 yrs); and 40 normal volunteers (32.8 ± 6.4 yrs) were recruited for this study. Data of 17 women were not used due to motion and other artifacts. Written informed consent was obtained and Institutional ethical committee approved the study. MR investigations were performed using a phased array breast matrix coil at 1.5 T (Siemens Avanto). Following the scout image, T2-weighted fat suppressed images in the transverse, sagittal and coronal planes and DCEMRI using 3D FLASH where-ever indicated was carried out. The in-vivo proton MRS using a single voxel PRESS sequence was carried out with water+lipid suppression using TR=1500 ms, TE=100 ms, averages=128, TA= 3.18 minutes. An additional spectrum of the same voxel without water and lipid suppression was obtained for the concentration calculation using water signal as an internal reference. The tCho concentration was calculated using the modified equation reported by Baik et al for 1.5 T (1). MRS experiments repeated in ten IDC patients at two different time periods showed that the difference in the coefficient of variation between the two measurements was 0.01. The mean difference of 95% confidence intervals was found to be 0.074 (-0.093 to 0.241), indicating that there was not much deviation between the two measurements. Tumor volume was measured using the formula: volume = ST (A1+A2...An). In 6 patients, ROI was drawn twice to find out intra-individual variation, which was verified latter by another person to check the inter-individual variation. The inter observer agreement was assessed using intra class correlation coefficient (ICC). The ICC was 0.99 indicating the better reproducibility of the volume measurements by two different observers. Statistical analyses were carried out using statistical software SPSS 16.0.

Results: Figure 1 shows the representative MR spectra obtained from a (A) malignant breast tissue of an LABC patient with IDC; (B) benign breast tissue and (C) from normal breast tissue of a volunteer. The concentration of tCho in EABC (5.4 ± 3.7 mmol/kg) and LABC patients (4.2 ± 2.3 mmol/kg) were significantly higher compared to benign lesions (1.62 ± 0.87 mmol/kg) and the normal breast tissue of controls (0.58 ± 0.36 mmol/kg);($p< 0.05$). Figure 2 represents the box plot showing the comparison of mean tCho concentration among malignant, benign and normal breast tissues. The tumor volume was found to be significant lower in EABC (7.1 ± 4.9 cm^3) compared to LABC patients (77.3 ± 74 cm^3). Further analysis showed that tCho concentration was significantly higher in stage IIA (5.4 ± 3.7 mmol/kg) patients (EBC) compared to LABC patients of stage III (A and B+C). A statistically significant difference was observed in tumor volume among various stages [$p<0.05$ between (IIA) and (IIIA); (IIA) and [(III) B+C]; (IIB) and (IIIA); (b) and (d)].

A cut-off value for the discrimination of normal, benign, EABC and LABC breast tissues was calculated using ROC analysis. Accordingly, a cut-off value of 2.54 mmol/kg (sensitivity 76%; specificity 75%; area under the curve 0.89) was obtained for the differentiation of malignant and benign lesions. Similarly, a cut-off value of 1.45 mmol/kg (sensitivity 93%; specificity 100%; area under the curve 0.99) and a cut-off value of 0.82 mmol/kg (sensitivity 73%; specificity 69%; area under the curve 0.82) for tCho were obtained for the differentiation of malignant and normal breast tissues and benign from normal breast tissues, respectively. A cut off value between benign and EABC was 2.58 mmol/kg with sensitivity of 80.8% and specificity of 80.8%; area under the curve 0.91 and between LABC and benign was 2.46 mmol/kg with sensitivity of 77.8% and specificity of 76.9%; area under the curve 0.89.

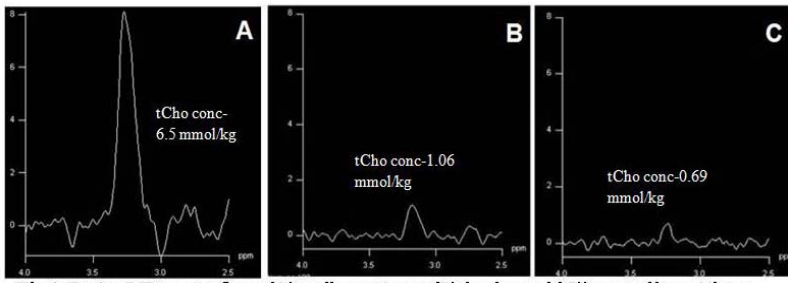

Fig-1-Proton MR spectra from (A) malignant tumor (B) benign and (C)) normal breast tissue

Discussion: In the present study we determined the absolute concentration of tCho in a large cohort of women using water as an internal reference and determined a cut-off value for tCho concentrations for the differentiation of EABC, LABC, benign and normal breast tissues. The elevation of the tCho in malignant cells is due to increased cellular replication and therefore increased synthesis of cellular membranes. EABC patients had statistically significantly higher tCho concentration compared to LABC patients. One of the possible reasons could be that as the tumor grows, its central region becomes necrotic due to non-uniform supply of the nutrient materials. The surrounding area consists of living cells as surface regions, receives more nutrients sufficient to undergo mitosis while cells in the region between the necrotic and the surface regions can get nutrients only to sustain life, but not enough to support their proliferation. Therefore, it may not be necessary for large tumors like LABC to have high tCho concentrations; however, it is possible that concentrations of tCho are dependent on tumor grade and cell differentiation. Our data indicated a wide range of tCho concentrations in the patient population studied. This might be attributed to the heterogeneous nature of the breast lesions or other molecular features of breast cancer. It is reported that detection of tCho was difficult in diffusive enhancement type cancers because of the intermingling of tumor cells with adipose tissue (2). A cut off value between benign and EABC was 2.58 mmol/kg with higher sensitivity of 80.8% and specificity of 80.8%) compared to the sensitivity of 77.8% and specificity of 76.9% obtained to differentiate LABC from benign for a cut off value of 2.46 mmol/kg. LABC patients of various stages showed larger tumor volumes compared to EABC patients. Further, the cut-off values determined to differentiate various breast tissues would help in providing the differential diagnosis of breast lesions and may help in the progress of breast proton MRS from the stage of investigational research to the role that could be efficaciously used in routine clinical practice.

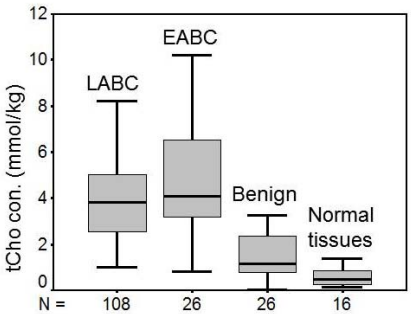

Figure 2: Box plot showing the comparison of mean tCho concentration (mmol/kg) among LABC, EABC, benign and normal breast tissues.

References: (1) Baik HM et al. MAGMA 2006; 19:96-104; (2) Baik HM, et al. Magn Reson Imaging 2008;26:523-31