Quantification of absolute concentration of choline for differentiation of malignant, benign and normal breast tissues by in-vivo proton MR spectroscopy.

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Objective: To determine the absolute concentration of choline containing compounds (tCho) for differentiation of malignant, benign and normal breast tissues using in- vivo proton (^{1}H) MRS at 1.5 T.

Introduction: Early detection of breast cancer provides significant improvements in the rate of cure of the disease, quality of life and overall survival of patients. Several studies reported that the presence of a total choline containing compounds (tCho) signal in the in-vivo MR spectrum of breast tissue can be used to differentiate the malignant from benign tumors and addition of in-vivo ^{1}H-MRS methodology improves the specificity of MRI (1-4). However, the technical challenges presented by a combination of relatively poor achievable B_o inhomogeneity and the requirement of lipid suppression have limited the progress of MRS in clinical settings. Further, recent studies showed that tCho was detected in benign lesions, questioning the diagnostic ability of qualitative MRS (2,5). Therefore, quantitative estimation of the absolute concentration of tCho in normal tissue as well as in malignant and benign pathologies is necessary. Thus, the aim of the present study was twofold: (a) to demonstrate the feasibility of using internal reference method for quantifying the tCho concentration, and (b) to compare the concentration values in malignant tissue of breast cancer patients, benign lesions and normal breast tissue of volunteers in a clinical setting.

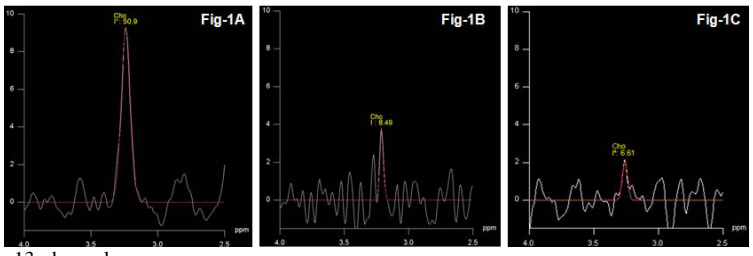
Material and Methods: A total of 87 women including 52 patients with cytologically proven infiltrating ductal carcinoma (44.3 ± 12.4, range 27 - 68 yrs), benign (n=15**;** 32.1 ± 8.0 range 16-38 yrs) attending the breast cancer clinic of our Institute and 20 normal volunteers (34.3 ± 10.3, range 21 - 42 yrs) with no breast pathology were recruited for the present study. Written informed consent was obtained and Institutional ethical committee approved the study. MR examinations were performed using a circularly polarized breast matrix coil at 1.5 T (MAGNETOM Avanto, Siemens Healthcare Sector, Germany). Tumor was localized using T1 and T2 weighted images with fat suppression in three 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 pulse sequence with water+lipid suppression using the following parameters: TR=1500 ms, TE=100 ms, averages=128, total acquisition time was 3:18 minutes. An additional spectrum from the same voxel without water and lipid suppression, was also obtained for concentration calculation using the water signal as internal reference. The following equation for the determination of the absolute concentration of the tCho signal was used as described in (1).

$$[Cho] = \frac{n_{H_2O}}{n_{Cho}\, MW_{H_2O}} \times \frac{S_{Cho}}{S_{H_2O}} \times \frac{f_{T_1 H_2O}}{f_{T_1 Cho}} \times \frac{f_{T_2 H_2O}}{f_{T_2 Cho}} \qquad [1],$$

$$f_{T_1} = 1 - \exp(-TR/T_1) \qquad [2],$$

$$f_{T_2} = \exp(-TE/T_2) \qquad [3]$$

where S_{Cho}: integral of choline signal, S_{H2O}: integral of internal water signal, n_{Cho}: number of contributing ^{1}H nuclei of choline, n_{H2O}: number of contributing 1H nuclei of water, MW_{H2O}: molecular weight of water in g/mol, f_{T1}: correction factor due to T1 values of choline/ water and TR of the sequence, f_{T2}: correction factor due to T2 values of choline/ water and TE of the sequence. Using the automated normalization with an internal water reference scan in the post-processing software, we used the relative normalized integral of choline (I*_int_Cho = S_{Cho} / S_{H2O}) directly in the formula below and calculated the absolute tCho concentration: [Cho] = (I*_int_Cho/100000)*8792.78 mmol/kg. One way ANOVA was used to compare the tCho concentration among patient groups and volunteers. A p-value of less than 0.05 was considered significant. Statistical analyses were carried out using SPSS 11.5 software.



Result: Figures 1(A-C) show the typical MR spectrum obtained from (A) breast cancer patient with infiltrating ductal carcinoma (malignant), (B) from benign lesion and (C) from normal breast tissue of a volunteer. The tCho peak was observed in all malignant lesions. Of the 15 benign lesions, 13 showed tCho and of 20 volunteers, tCho was observed in 8 volunteers. The mean concentration of tCho for malignant tumor was 4.04 ± 2.08 mmol/kg (range 1.02-10.8 mmol/kg) which was significantly higher compared to benign (n=13; 1.37 ± 0.8 mmol/kg) and normal breast tissues (n=8; 0.40 ± 0.24 mmol/kg) (Table 1; Fig. 2). The concentration of tCho between volunteers and benign lesions was not statistically significant.

Table 1: tCho concentration (mmol/Kg) (Mean ± SD)

Groups	Malignant (a)	Benign (b)	Vounteer (c)
Concentration	4.04±2.08 (n=52)	1.37±0.83 (n=13)	0.40±0.24 (n=8)
P-value	0.001(a with b and c)		

Discussion: The absolute quantification of tCho levels was carried out using internal reference method in breast cancer patients with malignant lesions and with benign lesions and normal tissue of volunteers. An interesting finding is that a composite tCho signal in ^{1}H spectra was observed in normal breast tissue (8/20) as well as in benign lesions (13/15). This has been pointed out as a limitation in the use of the composite Cho signal as a marker for breast cancer. Our study showed significantly higher concentration of tCho in patients with malignant tumors (range 1.02 to10.8 mmol/kg) compared to benign (range 0.04 to 2.3 mmol/kg) and normal tissues (range 0.16 to 0.87 mmol/kg) that clearly differentiates the malignant tissue from benign as well as from normal tissue. These results suggest that the accurate determination of tCho concentration would help in providing the unambiguous diagnosis of breast lesions. Roebuck et al (6) reported the tCho concentration in the range of 0.7-2.1 mM using external referencing method. However, Baik et al (1) reported large variation in the tCho concentration in the malignant lesions in range of 0.76 – 21.2 mmol/kg using water as an internal reference. The tCho concentration of malignant tissue observed in present study is consistent with these studies (1,6). Changes in tCho concentration after 24 hrs of the first dose of therapy have been reported by Meisamy et al (7). The use of internal reference method overcomes some of the limitations of the external reference method like the need for correction for partial volume effect and separate calibration experiments. Further measurements on more number of patients and volunteers would provide a cut-off value of tCho concentration that could be used for diagnosis of malignant tumor from various benign breast pathologies.

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